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(54) Title: **COMPLEMENTARY DNA'S ENCODING PROTEINS WITH SIGNAL PEPTIDES**

(57) Abstract: The sequences of cDNAs encoding secreted proteins are disclosed. The cDNAs can be used to express secreted proteins or fragments thereof or to obtain antibodies capable of specifically binding to the secreted proteins. The cDNAs may also be used in diagnostic, forensic, gene therapy, and chromosome mapping procedures. The cDNAs may also be used to design expression vectors and secretion vectors.

**COMPLEMENTARY DNA'S ENCODING PROTEINS WITH SIGNAL PEPTIDES**

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**Background of the Invention**

The estimated 50,000-100,000 genes scattered along the human chromosomes offer tremendous promise for the understanding, diagnosis, and treatment of human diseases. In addition, probes capable of specifically hybridizing to loci distributed throughout the human genome find applications in the construction of high resolution chromosome maps and in the identification of  
10 individuals.

In the past, the characterization of even a single human gene was a painstaking process, requiring years of effort. Recent developments in the areas of cloning vectors, DNA sequencing, and computer technology have merged to greatly accelerate the rate at which human genes can be isolated, sequenced, mapped, and characterized.

15 Currently, two different approaches are being pursued for identifying and characterizing the genes distributed along the human genome. In one approach, large fragments of genomic DNA are isolated, cloned, and sequenced. Potential open reading frames in these genomic sequences are identified using bio-informatics software. However, this approach entails sequencing large stretches of human DNA which do not encode proteins in order to find the protein encoding sequences  
20 scattered throughout the genome. In addition to requiring extensive sequencing, the bio-informatics software may mischaracterize the genomic sequences obtained, *i.e.*, labeling non-coding DNA as coding DNA and vice versa.

An alternative approach takes a more direct route to identifying and characterizing human genes. In this approach, complementary DNAs (cDNAs) are synthesized from isolated messenger  
25 RNAs (mRNAs) which encode human proteins. Using this approach, sequencing is only performed on DNA which is derived from protein coding fragments of the genome. Often, only short stretches of the cDNAs are sequenced to obtain sequences called expressed sequence tags (ESTs). The ESTs may then be used to isolate or purify cDNAs which include sequences adjacent to the EST sequences. The cDNAs may contain all of the sequence of the EST which was used to obtain them  
30 or only a fragment of the sequence of the EST which was used to obtain them. In addition, the cDNAs may contain the full coding sequence of the gene from which the EST was derived or, alternatively, the cDNAs may include fragments of the coding sequence of the gene from which the EST was derived. It will be appreciated that there may be several cDNAs which include the EST sequence as a result of alternate splicing or the activity of alternative promoters.

35 In the past, these short EST sequences were often obtained from oligo-dT primed cDNA libraries. Accordingly, they mainly corresponded to the 3' untranslated region of the mRNA. In part, the prevalence of EST sequences derived from the 3' end of the mRNA is a result of the fact that typical techniques for obtaining cDNAs, are not well suited for isolating cDNA sequences

derived from the 5' ends of mRNAs (Adams *et al.*, *Nature* 377:3-174, 1996, Hillier *et al.*, *Genome Res.* 6:807-828, 1996). In addition, in those reported instances where longer cDNA sequences have been obtained, the reported sequences typically correspond to coding sequences and do not include the full 5' untranslated region (5'UTR) of the mRNA from which the cDNA is derived. Indeed, 5'UTRs have been shown to affect either the stability or translation of mRNAs. Thus, regulation of gene expression may be achieved through the use of alternative 5'UTRs as shown, for instance, for the translation of the tissue inhibitor of metalloprotease mRNA in mitogenically activated cells (Waterhouse *et al.*, *J Biol Chem.* 265:5585-9, 1990). Furthermore, modification of 5'UTR through mutation, insertion or translocation events may even be implied in pathogenesis. For instance, the fragile X syndrome, the most common cause of inherited mental retardation, is partly due to an insertion of multiple CGG trinucleotides in the 5'UTR of the fragile X mRNA resulting in the inhibition of protein synthesis via ribosome stalling (Feng *et al.*, *Science* 268:731-4, 1995). An aberrant mutation in regions of the 5'UTR known to inhibit translation of the proto-oncogene *c-myc* was shown to result in upregulation of c-myc protein levels in cells derived from patients with multiple myelomas (Willis *et al.*, *Curr Top Microbiol Immunol* 224:269-76, 1997). In addition, the use of oligo-dT primed cDNA libraries does not allow the isolation of complete 5'UTRs since such incomplete sequences obtained by this process may not include the first exon of the mRNA, particularly in situations where the first exon is short. Furthermore, they may not include some exons, often short ones, which are located upstream of splicing sites. Thus, there is a need to obtain sequences derived from the 5' ends of mRNAs.

Moreover, despite the great amount of EST data that large-scale sequencing projects have yielded (Adams *et al.*, *Nature* 377:174, 1996, Hillier *et al.*, *Genome Res.* 6:807-828, 1996), information concerning the biological function of the mRNAs corresponding to such obtained cDNAs has revealed to be limited. Indeed, whereas the knowledge of the complete coding sequence is absolutely necessary to investigate the biological function of mRNAs, ESTs yield only partial coding sequences. So far, large-scale full-length cDNA cloning has been achieved only with limited success because of the poor efficiency of methods for constructing full-length cDNA libraries. Indeed, such methods require either a large amount of mRNA (Ederly *et al.*, 1995), thus resulting in non representative full-length libraries when small amounts of tissue are available or require PCR amplification (Maruyama *et al.*, 1994; CLONTECHniques, 1996) to obtain a reasonable number of clones, thus yielding strongly biased cDNA libraries where rare and long cDNAs are lost. Thus, there is a need to obtain full-length cDNAs, *i.e.* cDNAs containing the full coding sequence of their corresponding mRNAs.

While many sequences derived from human chromosomes have practical applications, approaches based on the identification and characterization of those chromosomal sequences which encode a protein product are particularly relevant to diagnostic and therapeutic uses. Of the 50,000-100,000 protein coding genes, those genes encoding proteins which are secreted from the cell in

which they are synthesized, as well as the secreted proteins themselves, are particularly valuable as potential therapeutic agents. Such proteins are often involved in cell to cell communication and may be responsible for producing a clinically relevant response in their target cells. In fact, several secretory proteins, including tissue plasminogen activator, G-CSF, GM-CSF, erythropoietin, human  
5 growth hormone, insulin, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , and interleukin-2, are currently in clinical use. These proteins are used to treat a wide range of conditions, including acute myocardial infarction, acute ischemic stroke, anemia, diabetes, growth hormone deficiency, hepatitis, kidney carcinoma, chemotherapy induced neutropenia and multiple sclerosis. For these reasons, cDNAs encoding secreted proteins or fragments thereof represent a particularly valuable source of  
10 therapeutic agents. Thus, there is a need for the identification and characterization of secreted proteins and the nucleic acids encoding them.

In addition to being therapeutically useful themselves, secretory proteins include short peptides, called signal peptides, at their amino termini which direct their secretion. These signal peptides are encoded by the signal sequences located at the 5' ends of the coding sequences of genes  
15 encoding secreted proteins. Because these signal peptides will direct the extracellular secretion of any protein to which they are operably linked, the signal sequences may be exploited to direct the efficient secretion of any protein by operably linking the signal sequences to a gene encoding the protein for which secretion is desired. In addition, fragments of the signal peptides called membrane-translocating sequences, may also be used to direct the intracellular import of a peptide or  
20 protein of interest. This may prove beneficial in gene therapy strategies in which it is desired to deliver a particular gene product to cells other than the cells in which it is produced. Signal sequences encoding signal peptides also find application in simplifying protein purification techniques. In such applications, the extracellular secretion of the desired protein greatly facilitates purification by reducing the number of undesired proteins from which the desired protein must be  
25 selected. Thus, there exists a need to identify and characterize the 5' fragments of the genes for secretory proteins which encode signal peptides.

Sequences coding for secreted proteins may also find application as therapeutics or diagnostics. In particular, such sequences may be used to determine whether an individual is likely to express a detectable phenotype, such as a disease, as a consequence of a mutation in the coding  
30 sequence for a secreted protein. In instances where the individual is at risk of suffering from a disease or other undesirable phenotype as a result of a mutation in such a coding sequence, the undesirable phenotype may be corrected by introducing a normal coding sequence using gene therapy. Alternatively, if the undesirable phenotype results from overexpression of the protein encoded by the coding sequence, expression of the protein may be reduced using antisense or triple  
35 helix based strategies.

The secreted human polypeptides encoded by the coding sequences may also be used as therapeutics by administering them directly to an individual having a condition, such as a disease,



resulting from a mutation in the sequence encoding the polypeptide. In such an instance, the condition can be cured or ameliorated by administering the polypeptide to the individual.

In addition, the secreted human polypeptides or fragments thereof may be used to generate antibodies useful in determining the tissue type or species of origin of a biological sample. The antibodies may also be used to determine the cellular localization of the secreted human polypeptides or the cellular localization of polypeptides which have been fused to the human polypeptides. In addition, the antibodies may also be used in immunoaffinity chromatography techniques to isolate, purify, or enrich the human polypeptide or a target polypeptide which has been fused to the human polypeptide.

Public information on the number of human genes for which the promoters and upstream regulatory regions have been identified and characterized is quite limited. In part, this may be due to the difficulty of isolating such regulatory sequences. Upstream regulatory sequences such as transcription factor binding sites are typically too short to be utilized as probes for isolating promoters from human genomic libraries. Recently, some approaches have been developed to isolate human promoters. One of them consists of making a CpG island library (Cross *et al.*, *Nature Genetics* 6: 236-244, 1994). The second consists of isolating human genomic DNA sequences containing SpeI binding sites by the use of SpeI binding protein. (Mortlock *et al.*, *Genome Res.* 6:327-335, 1996). Both of these approaches have their limits due to a lack of specificity and of comprehensiveness. Thus, there exists a need to identify and systematically characterize the 5' fragments of the genes.

cDNAs including the 5' ends of their corresponding mRNA may be used to efficiently identify and isolate 5'UTRs and upstream regulatory regions which control the location, developmental stage, rate, and quantity of protein synthesis, as well as the stability of the mRNA (Theil *et al.*, *BioFactors* 4:87-93, (1993). Once identified and characterized, these regulatory regions may be utilized in gene therapy or protein purification schemes to obtain the desired amount and locations of protein synthesis or to inhibit, reduce, or prevent the synthesis of undesirable gene products.

In addition, cDNAs containing the 5' ends of secretory protein genes may include sequences useful as probes for chromosome mapping and the identification of individuals. Thus, there is a need to identify and characterize the sequences upstream of the 5' coding sequences of genes encoding secretory proteins.

### **Summary of the Invention**

The present invention relates to purified, isolated, or recombinant cDNAs which encode secreted proteins or fragments thereof. Preferably, the purified, isolated or recombinant cDNAs contain the entire open reading frame of their corresponding mRNAs, including a start codon and a stop codon. For example, the cDNAs may include nucleic acids encoding the signal peptide as well as the mature protein. Such cDNAs will be referred herein as "full-length" cDNAs. Alternatively,

the cDNAs may contain a fragment of the open reading frame. Such cDNAs will be referred herein as "ESTs" or "5'ESTs". In some embodiments, the fragment may encode only the sequence of the mature protein. Alternatively, the fragment may encode only a fragment of the mature protein. A further aspect of the present invention is a nucleic acid which encodes the signal peptide of a  
5 secreted protein.

The term "corresponding mRNA" refers to the mRNA which was the template for the cDNA synthesis which produced the cDNA of the present invention.

As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. Purification of starting material or natural material is at least one order of magnitude,  
10 preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. As an example, purification from 0.1 % concentration to 10 % concentration is two orders of magnitude.

To illustrate, individual cDNA clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. The sequences obtained from these clones could not be  
15 obtained directly either from the library or from total human DNA. The cDNA clones are not naturally occurring as such, but rather are obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The conversion of mRNA into a cDNA library involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection. Thus, creating a cDNA library from messenger RNA and subsequently isolating  
20 individual clones from that library results in an approximately  $10^4$ - $10^6$  fold purification of the native message.

The term "purified" is further used herein to describe a polypeptide or polynucleotide of the invention which has been separated from other compounds including, but not limited to, polypeptides or polynucleotides, carbohydrates, lipids, etc. The term "purified" may be used to specify the separation of  
25 monomeric polypeptides of the invention from oligomeric forms such as homo- or hetero- dimers, trimers, etc. The term "purified" may also be used to specify the separation of covalently closed polynucleotides from linear polynucleotides. A polynucleotide is substantially pure when at least about 50%, preferably 60 to 75% of a sample exhibits a single polynucleotide sequence and conformation (linear versus covalently close). A substantially pure polypeptide or polynucleotide typically comprises  
30 about 50%, preferably 60 to 90% weight/weight of a polypeptide or polynucleotide sample, respectively, more usually about 95%, and preferably is over about 99% pure. Polypeptide and polynucleotide purity, or homogeneity, is indicated by a number of means well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single band upon staining the gel. For certain purposes higher resolution can be provided by using HPLC or other means well known  
35 in the art. As an alternative embodiment, purification of the polypeptides and polynucleotides of the present invention may be expressed as "at least" a percent purity relative to heterologous polypeptides and polynucleotides (DNA, RNA or both). As a preferred embodiment, the polypeptides and

polynucleotides of the present invention are at least; 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 96%, 98%, 99%, or 100% pure relative to heterologous polypeptides and polynucleotides, respectively. As a further preferred embodiment the polypeptides and polynucleotides have a purity ranging from any number, to the thousandth position, between 90% and 100% (e.g., a polypeptide or  
5 polynucleotide at least 99.995% pure) relative to either heterologous polypeptides or polynucleotides, respectively, or as a weight/weight ratio relative to all compounds and molecules other than those existing in the carrier. Each number representing a percent purity, to the thousandth position, may be claimed as individual species of purity.

As used herein, the term "recombinant polynucleotide" means that the cDNA is adjacent to  
10 "backbone" nucleic acid to which it is not adjacent in its natural environment. Additionally, to be "enriched" the cDNAs will represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid backbone molecules. Backbone molecules according to the present invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids, and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest.  
15 Preferably, the enriched cDNAs represent 15% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. More preferably, the enriched cDNAs represent 50% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. In a highly preferred embodiment, the enriched cDNAs represent 90% or more (including any number between 90 and 100%, to the thousandth position, e.g., 99.5%) # of the number of nucleic acid inserts in  
20 the population of recombinant backbone molecules.

Unless otherwise specified, nucleotides and amino acids of polynucleotide and polypeptide fragments (respectively) of the present invention are contiguous and not interrupted by heterologous sequences.

The term "isolated" requires that the material be removed from its original environment (e. g.,  
25 the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural  
30 environment. Specifically excluded from the definition of "isolated" are: naturally occurring chromosomes (such as chromosome spreads), artificial chromosome libraries, genomic libraries, and cDNA libraries that exist either as an in vitro nucleic acid preparation or as a transfected/transformed host cell preparation, wherein the host cells are either an in vitro heterogeneous preparation or plated as a heterogeneous population of single colonies, and/or further wherein the polynucleotide of the present  
35 invention makes up less than 5% (or alternatively 1%, 2%, 3%, 4%, 10%, 25%, 50%, 75%, or 90%, 95%, or 99%) of the number of nucleic acid inserts in the vector molecules. Further specifically excluded are whole cell genomic DNA or whole cell RNA preparations (including said whole cell

preparations which are mechanically sheared or enzymatically digested). Further specifically excluded are the above whole cell preparations as either an in vitro preparation or as a heterogeneous mixture separated by electrophoresis (including blot transfers of the same) wherein the polynucleotide of the invention have not been further separated from the heterologous polynucleotides in the electrophoresis medium (e.g., further separating by excising a single band from a heterogeneous band population in an agarose gel or nylon blot).

Thus, cDNAs encoding secreted polypeptides or fragments thereof which are present in cDNA libraries in which one or more cDNAs encoding secreted polypeptides or fragments thereof make up 5% or more of the number of nucleic acid inserts in the backbone molecules are "enriched recombinant cDNAs" as defined herein. Likewise, cDNAs encoding secreted polypeptides or fragments thereof which are in a population of plasmids in which one or more cDNAs of the present invention have been inserted such that they represent 5% or more of the number of inserts in the plasmid backbone are "enriched recombinant cDNAs" as defined herein. However, cDNAs encoding secreted polypeptides or fragments thereof which are in cDNA libraries in which the cDNAs encoding secreted polypeptides or fragments thereof constitute less than 5% of the number of nucleic acid inserts in the population of backbone molecules, such as libraries in which backbone molecules having a cDNA insert encoding a secreted polypeptide are extremely rare, are not "enriched recombinant cDNAs."

The term "polypeptide" refers to a polymer of amino acids without regard to the length of the polymer; thus, "peptides," "oligopeptides", and "proteins" are included within the definition of polypeptide and used interchangeably herein. This term also does not specify or exclude chemical or post-expression modifications of the polypeptides of the invention, although chemical or post-expression modifications of these polypeptides may be included or excluded as specific embodiments. Therefore, for example, modifications to polypeptides that include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups and the like are expressly encompassed by the term polypeptide. Further, polypeptides with these modifications may be specified as individual species to be included or excluded from the present invention. The natural or other chemical modifications, such as those listed in examples above can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of

pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS -  
 5 STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12, 1983; Seifter et al., Meth Enzymol 182:626-646, 1990; Rattan et al., Ann NY Acad Sci 663:48-62, 1992). Also included within the definition are polypeptides which contain one or more analogs of an amino acid  
 10 (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. The term "polypeptide" may also be used interchangeably with the term "protein".

15 As used interchangeably herein, the terms "nucleic acid molecule", "oligonucleotides", and "polynucleotides" include RNA or, DNA (either single or double stranded, coding, non-coding, complementary or antisense), or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form (although each of the above species may be particularly specified). The term "nucleotide" as used herein as an adjective to describe molecules comprising RNA, DNA, or  
 20 RNA/DNA hybrid sequences of any length in single-stranded or duplex form. The term "nucleotide" is also used herein as a noun to refer to individual nucleotides or varieties of nucleotides, meaning a molecule, or individual unit in a larger nucleic acid molecule, comprising a purine or pyrimidine, a ribose or deoxyribose sugar moiety, and a phosphate group, or phosphodiester linkage in the case of nucleotides within an oligonucleotide or polynucleotide. The term "nucleotide" is also used herein  
 25 to encompass "modified nucleotides" which comprise at least one modifications (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar; for examples of analogous linking groups, purine, pyrimidines, and sugars see for example PCT publication No. WO 95/04064. Preferred modifications of the present invention include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil,  
 30 hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-  
 35 D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v) ybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester,

uracil-5-oxyacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Methylenemethylimino linked oligonucleosides as well as mixed backbone compounds having, may be prepared as described in U.S. Pat. Nos. 5,378,825; 5,386,023; 5,489,677; 5,602,240; and 5,610,289. Formacetal and thioformacetal linked oligonucleosides may be prepared  
5 as described in U.S. Pat. Nos. 5,264,562 and 5,264,564. Ethylene oxide linked oligonucleosides may be prepared as described in U.S. Pat. No. 5,223,618. Phosphinate oligonucleotides may be prepared as described in U.S. Pat. No. 5,508,270. Alkyl phosphonate oligonucleotides may be prepared as described in U.S. Pat. No. 4,469,863. 3'-Deoxy-3'-methylene phosphonate oligonucleotides may be prepared as described in U.S. Pat. Nos. 5,610,289 or 5,625,050. Phosphoramidite oligonucleotides  
10 may be prepared as described in U.S. Pat. No. 5,256,775 or U.S. Pat. No. 5,366,878. Alkylphosphonothioate oligonucleotides may be prepared as described in published PCT applications WO 94/17093 and WO 94/02499. 3'-Deoxy-3'-amino phosphoramidate oligonucleotides may be prepared as described in U.S. Pat. No. 5,476,925. Phosphotriester oligonucleotides may be prepared as described in U.S. Pat. No. 5,023,243. Borano phosphate  
15 oligonucleotides may be prepared as described in U.S. Pat. Nos. 5,130,302 and 5,177,198.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300kb, 200kb, 100kb, 50kb, 10kb, 7.5kb, 5kb, 2.5kb, 2kb, 1.5kb, or 1kb in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding  
20 sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 75, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

25 The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, *ex vivo* generation, or a combination thereof, as well as utilizing any purification methods known in the art.

The terms "comprising", "consisting of" and "consisting essentially of" may be interchanged for one another throughout the instant application". The term "having" has the same meaning as  
30 "comprising" and may be replaced with either the term "consisting of" or "consisting essentially of".

"Stringent", "moderate," and "low" hybridization conditions are as defined below.

A sequence which is "operably linked" to a regulatory sequence such as a promoter means that said regulatory element is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the nucleic acid of interest. As used herein, the  
35 term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence.

The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein to refer to nucleotides which can be hydrogen bonded to one another by virtue of their sequence identities in a manner like that found in double-helical DNA with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three 5 hydrogen bonds (See Stryer, L., *Biochemistry*, 4<sup>th</sup> edition, 1995).

The terms "complementary" or "complement thereof" are used herein to refer to the sequences of polynucleotides which are capable of forming Watson & Crick base pairing with another specified polynucleotide throughout the entirety of the complementary region. For the purpose of the present invention, a first polynucleotide is deemed to be complementary to a second 10 polynucleotide when each base in the first polynucleotide is paired with its complementary base. Complementary bases are, generally, A and T (or A and U), or C and G. "Complement" is used herein as a synonym from "complementary polynucleotide," "complementary nucleic acid" and "complementary nucleotide sequence". These terms are applied to pairs of polynucleotides based solely upon their sequences and not any particular set of conditions under which the two 15 polynucleotides would actually bind. Preferably, a "complementary" sequence is a sequence which has an A at each position where there is a T on the opposite strand, a T at each position where there is an A on the opposite strand, a G at each position where there is a C on the opposite strand and a C at each position where there is a G on the opposite strand.

The term "allele" is used herein to refer to variants of a nucleotide sequence. A biallelic 20 polymorphism has two forms. Diploid organisms may be homozygous or heterozygous for an allelic form. Unless otherwise specified, the polynucleotides of the present invention encompass all allelic variants of the disclosed polynucleotides.

The term "upstream" is used herein to refer to a location that is toward the 5' end of the polynucleotide from a specific reference point.

25 As used herein, the term "non-human animal" refers to any non-human vertebrate animal, including insects, birds, rodents and more usually mammals. Preferred non-human animals include: primates; farm animals such as swine, goats, sheep, donkeys, cattle, horses, chickens, rabbits; and rodents, more preferably rats or mice. As used herein, the term "animal" is used to refer to any species in the animal kingdom, preferably vertebrates, including birds and fish, and more preferable 30 a mammal. Both the terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

The terms "vertebrate nucleic acid" and "vertebrate polypeptide" are used herein to refer to any nucleic acid or polypeptide respectively which are derived from a vertebrate species including birds and more usually mammals, preferably primates such as humans, farm animals such as swine, goats, 35 sheep, donkeys, and horses, rabbits or rodents, more preferably rats or mice. As used herein, the term "vertebrate" is used to refer to any vertebrate, preferably a mammal. The term "vertebrate" expressly embraces human subjects unless preceded with the term "non-human"

"Stringent", "moderate," and "low" hybridization conditions are as defined below.

The term "capable of hybridizing to the polyA tail of said mRNA" refers to and embraces all primers containing stretches of thymidine residues, so-called oligo(dT) primers, that hybridize to the 3' end of eukaryotic poly(A)+ mRNAs to prime the synthesis of a first cDNA strand. Techniques for  
5 generating said oligo(dT) primers and hybridizing them to mRNA to subsequently prime the reverse transcription of said hybridized mRNA to generate a first cDNA strand are well known to those skilled in the art and are described in *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. 1997 and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989, the entire disclosures of which are incorporated herein by reference.  
10 Preferably, said oligo(dT) primers are present in a large excess in order to allow the hybridization of all mRNA 3'ends to at least one oligo(dT) molecule. The priming and reverse transcription step are preferably performed between 37°C and 55°C depending on the type of reverse transcriptase used.

Preferred oligo(dT) primers for priming reverse transcription of mRNAs are oligonucleotides containing a stretch of thymidine residues of sufficient length to hybridize specifically to the polyA tail  
15 of mRNAs, preferably of 12 to 18 thymidine residues in length. More preferably, such oligo(T) primers comprise an additional sequence upstream of the poly(dT) stretch in order to allow the addition of a given sequence to the 5'end of all first cDNA strands which may then be used to facilitate subsequent manipulation of the cDNA. Preferably, this added sequence is 8 to 60 residues in length. For instance, the addition of a restriction site in 5' of cDNAs facilitates subcloning of the obtained cDNA.  
20 Alternatively, such an added 5'end may also be used to design primers of PCR to specifically amplify cDNA clones of interest.

In particular, the present invention relates to cDNAs which were derived from genes encoding secreted proteins. As used herein, a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of  
25 signal peptides in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g. soluble proteins), or partially (e.g. receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum.

cDNAs encoding secreted proteins may include nucleic acid sequences, called signal  
30 sequences, which encode signal peptides which direct the extracellular secretion of the proteins encoded by the cDNAs. Generally, the signal peptides are located at the amino termini of secreted proteins. Polypeptides comprising these signal peptides (as delineated in the sequence listing), and polynucleotides encoding the same, are preferred embodiments of the present invention.

Secreted proteins are translated by ribosomes associated with the "rough" endoplasmic  
35 reticulum. Generally, secreted proteins are co-translationally transferred to the membrane of the endoplasmic reticulum. Association of the ribosome with the endoplasmic reticulum during translation of secreted proteins is mediated by the signal peptide. The signal peptide is typically



cleaved following its co-translational entry into the endoplasmic reticulum. After delivery to the endoplasmic reticulum, secreted proteins may proceed through the Golgi apparatus. In the Golgi apparatus, the proteins may undergo post-translational modification before entering secretory vesicles which transport them across the cell membrane.

5           The cDNAs of the present invention have several important applications. For example, they may be used to express the entire secreted protein which they encode. Alternatively, they may be used to express fragments of the secreted protein. The fragments may comprise the signal peptides encoded by the cDNAs or the mature proteins encoded by the cDNAs (i.e. the proteins generated when the signal peptide is cleaved off). The cDNAs and fragments thereof also have  
10 important applications as polynucleotides. For example, the cDNAs of the sequence listing and fragments thereof, may be used to distinguish human tissues/cells from non-human tissues/cells and to distinguish between human tissues/cells that do and do not express the polynucleotides comprising the cDNAs. By knowing the tissue expression pattern of the cDNAs, either through routine experimentation or by using the instant disclosure, the polynucleotides of the present invention may be  
15 used in methods of determining the identity of an unknown tissue/cell sample. As part of determining the identity of an unknown tissue/cell sample, the polynucleotides of the present invention may be used to determine what the unknown tissue/cell sample is and what the unknown sample is not. For example, if a cDNA is expressed in a particular tissue/cell type, and the unknown tissue/cell sample does not express the cDNA, it may be inferred that the unknown tissue/cells are either not human or not the same  
20 human tissue/cell type as that which expresses the cDNA. These methods of determining tissue/cell identity are based on methods which detect the presence or absence of the mRNA (or corresponding cDNA) in a tissue/cell sample using methods well known in the art (e.g., hybridization or PCR based methods).

In other useful applications, fragments of the cDNAs encoding signal peptides as well as  
25 degenerate polynucleotides encoding the same, may be ligated to sequences encoding either the polypeptide from the same gene or to sequences encoding a heterologous polypeptide to facilitate secretion.

Antibodies which specifically recognize the entire secreted proteins encoded by the cDNAs or fragments thereof having at least 6 consecutive amino acids, 8 consecutive amino acids, 10  
30 consecutive amino acids, at least 15 consecutive amino acids, at least 25 consecutive amino acids, or at least 40 consecutive amino acids may also be obtained as described below. Antibodies which specifically recognize the mature protein generated when the signal peptide is cleaved may also be obtained as described below. Similarly, antibodies which specifically recognize the signal peptides encoded by the cDNAs may also be obtained.

35           In some embodiments, the cDNAs include the signal sequence. In other embodiments, the cDNAs may include the full coding sequence for the mature protein (i.e. the protein generated when the signal polypeptide is cleaved off). In addition, the cDNAs may include regulatory regions

upstream of the translation start site or downstream of the stop codon which control the amount, location, or developmental stage of gene expression. As discussed above, secreted proteins are therapeutically important. Thus, the proteins expressed from the cDNAs may be useful in treating or controlling a variety of human conditions. The cDNAs may also be used to obtain the corresponding  
5 genomic DNA. The term "corresponding genomic DNA" refers to the genomic DNA which encodes mRNA which includes the sequence of one of the strands of the cDNA in which thymidine residues in the sequence of the cDNA are replaced by uracil residues in the mRNA.

The cDNAs or genomic DNAs obtained therefrom may be used in forensic procedures to identify individuals or in diagnostic procedures to identify individuals having genetic diseases  
10 resulting from abnormal expression of the genes corresponding to the cDNAs. In addition, the present invention is useful for constructing a high resolution map of the human chromosomes.

The present invention also relates to secretion vectors capable of directing the secretion of a protein of interest. Such vectors may be used in gene therapy strategies in which it is desired to produce a gene product in one cell which is to be delivered to another location in the body.  
15 Secretion vectors may also facilitate the purification of desired proteins.

The present invention also relates to expression vectors capable of directing the expression of an inserted gene in a desired spatial or temporal manner or at a desired level. Such vectors may include sequences upstream of the cDNAs such as promoters or upstream regulatory sequences.

In addition, the present invention may also be used for gene therapy to control or treat  
20 genetic diseases. Signal peptides may also be fused to heterologous proteins to direct their extracellular secretion.

One embodiment of the present invention is a purified or isolated nucleic acid comprising the sequence of one of SEQ ID NOs: 24-73 or a sequence complementary thereto, allelic variants thereof, and degenerate variants thereof. In one aspect of this embodiment, the nucleic acid is  
25 recombinant.

Another embodiment of the present invention is a purified or isolated nucleic acid comprising at least 8 consecutive bases of the sequence of one of SEQ ID NOs: 24-73 or one of the sequences complementary thereto, allelic variants thereof, and degenerate variants thereof. In one aspect of this embodiment, the nucleic acid comprises at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40,  
30 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 consecutive bases of one of the sequences of SEQ ID NOs: 24-73 or one of the sequences complementary thereto, allelic variants thereof, and degenerate variants thereof. The nucleic acid may be a recombinant nucleic acid.

In addition to the above preferred nucleic acid sizes, further preferred sub-genuses of nucleic acids comprise at least 8 nucleotides, wherein "at least 8" is defined as any integer between 8 and the  
35 integer representing the 3' most nucleotide position as set forth in the sequence listing or elsewhere herein. Further included as preferred polynucleotides of the present invention are nucleic acid fragments at least 8 nucleotides in length, as described above, that are further specified in terms of

their 5' and 3' position. The 5' and 3' positions are represented by the position numbers set forth in the sequence listing below. For allelic and degenerate variants, position 1 is defined as the 5' most nucleotide of the ORF, i.e., the nucleotide "A" of the start codon with the remaining nucleotides numbered consecutively. Therefore, every combination of a 5' and 3' nucleotide position that a polynucleotide fragment of the present invention, at least 8 contiguous nucleotides in length, could occupy is included in the invention as an individual species. The polynucleotide fragments specified by 5' and 3' positions can be immediately envisaged and are therefore not individually listed solely for the purpose of not unnecessarily lengthening the specifications.

It is noted that the above species of polynucleotide fragments of the present invention may alternatively be described by the formula "a to b"; where "x" equals the 5' most nucleotide position and "y" equals the 3' most nucleotide position of the polynucleotide; and further where "x" equals an integer between 1 and the number of nucleotides of the polynucleotide sequence of the present invention minus 8, and where "y" equals an integer between 9 and the number of nucleotides of the polynucleotide sequence of the present invention; and where "x" is an integer smaller than "y" by at least 8.

The present invention also provides for the exclusion of any species of polynucleotide fragments of the present invention specified by 5' and 3' positions or sub-genuses of polynucleotides specified by size in nucleotides as described above. Any number of fragments specified by 5' and 3' positions or by size in nucleotides, as described above, may be excluded.

Another embodiment of the present invention is a vertebrate purified or isolated nucleic acid of at least 15, 18, 20, 23, 25, 28, 30, 35, 40, 50, 75, 100, 200, 300, 500 or 1000 nucleotides in length which hybridizes under stringent conditions to the sequence of one of SEQ ID NOs: 24-73 or a sequence complementary to one of the sequences of SEQ ID NOs: 24-73. In one aspect of this embodiment, the nucleic acid is recombinant.

Another embodiment of the present invention is a purified or isolated nucleic acid comprising the full coding sequences of one of SEQ ID NOs: 24-73, or an allelic variant thereof, wherein the full coding sequence optionally comprises the sequence encoding signal peptide as well as the sequence encoding mature protein. In one aspect of this embodiment, the nucleic acid is recombinant.

A further embodiment of the present invention is a purified or isolated nucleic acid comprising the nucleotides of one of SEQ ID NOs: 24-73, or an allelic variant thereof which encode a mature protein. In one aspect of this embodiment, the nucleic acid is recombinant. In another aspect of this embodiment, the nucleic acid is an expression vector wherein said nucleotides of one of SEQ ID NOs: 24-73, or an allelic variant thereof which encode a mature protein, are operably linked to a promoter.

Yet another embodiment of the present invention is a purified or isolated nucleic acid comprising the nucleotides of one of SEQ ID NOs: 24-73, or an allelic variant thereof, which encode

the signal peptide. In one aspect of this embodiment, the nucleic acid is recombinant. In another aspect of this embodiment, the nucleic acid is an fusion vector wherein said nucleotides of one of SEQ ID NOs: 24-73, or an allelic variant thereof which encode the signal peptide, are operably linked to a second nucleic acid encoding an heterologous polypeptide.

5 Another embodiment of the present invention is a purified or isolated nucleic acid encoding a polypeptide comprising the sequence of one of the sequences of SEQ ID NOs: 74-123, or allelic variant thereof. In one aspect of this embodiment, the nucleic acid is recombinant.

Another embodiment of the present invention is a purified or isolated nucleic acid encoding a polypeptide comprising the sequence of a mature protein included in one of the sequences of SEQ  
10 ID NOs: 74-123, or allelic variant thereof. In one aspect of this embodiment, the nucleic acid is recombinant.

Another embodiment of the present invention is a purified or isolated nucleic acid encoding a polypeptide comprising the sequence of a signal peptide included in one of the sequences of SEQ ID NOs: 74-123, or allelic variant thereof. In one aspect of this embodiment, the nucleic acid is  
15 recombinant. In another aspect it is present in a vector of the invention.

Further embodiments of the invention include isolated polynucleotides that comprise, a nucleotide sequence at least 70% identical, more preferably at least 75% identical, and still more preferably at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any of the polynucleotides of the present invention. Methods of determining identity include those well known  
20 in the art and described herein.

Yet another embodiment of the present invention is a purified or isolated protein comprising the sequence of one of SEQ ID NOs: 74-123, or allelic variant thereof.

Another embodiment of the present invention is a purified or isolated polypeptide comprising at least 5 or 8 consecutive amino acids of one of the sequences of SEQ ID NOs: 74-123.  
25 In one aspect of this embodiment, the purified or isolated polypeptide comprises at least 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 consecutive amino acids of one of the sequences of SEQ ID NOs: 74-123.

In addition to the above polypeptide fragments, further preferred sub-genuses of polypeptides comprise at least 8 amino acids, wherein "at least 8" is defined as any integer between 8 and the  
30 integer representing the C-terminal amino acid of the polypeptide of the present invention including the polypeptide sequences of the sequence listing below. Further included are species of polypeptide fragments at least 8 amino acids in length, as described above, that are further specified in terms of their N-terminal and C-terminal positions. Preferred species of polypeptide fragments specified by their N-terminal and C-terminal positions include the signal peptides delineated in the sequence  
35 listing below. However, included in the present invention as individual species are all polypeptide fragments, at least 8 amino acids in length, as described above, and may be particularly specified by a N-terminal and C-terminal position. That is, every combination of a N-terminal and C-terminal

position that a fragment at least 8 contiguous amino acid residues in length could occupy, on any given amino acid sequence of the sequence listing or of the present invention is included in the present invention

The present invention also provides for the exclusion of any fragment species specified by  
5 N-terminal and C-terminal positions or of any fragment sub-genus specified by size in amino acid residues as described above. Any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may be excluded as individual species.

The above polypeptide fragments of the present invention can be immediately envisaged  
10 using the above description and are therefore not individually listed solely for the purpose of not unnecessarily lengthening the specification. Moreover, the above fragments need not be active since they would be useful, for example, in immunoassays, in epitope mapping, epitope tagging, as vaccines, and as molecular weight markers. The above fragments may also be used to generate antibodies to a particular portion of the polypeptide. These antibodies can then be used in  
15 immunoassays well known in the art to distinguish between human and non-human cells and tissues or to determine whether cells or tissues in a biological sample are or are not of the same type which express the polypeptide of the present invention. Preferred polypeptide fragments of the present invention comprising a signal peptide may be used to facilitate secretion of either the polypeptide of the same gene or a heterologous polypeptide using methods well known in the art.

20 Another embodiment of the present invention is an isolated or purified polypeptide comprising a signal peptide of one of the polypeptides of SEQ ID NOs: 74-123.

Yet another embodiment of the present invention is an isolated or purified polypeptide comprising a mature protein of one of the polypeptides of SEQ ID NOs: 74-123.

Yet another embodiment of the present invention is an isolated or purified polypeptide  
25 comprising a full length polypeptide, mature protein, or signal peptide encoded by an allelic variant of the polynucleotides of the present invention.

A further embodiment of the present invention are polypeptides having an amino acid sequence with at least 70% similarity, and more preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to a polypeptide of the present invention, as well as polypeptides  
30 having an amino acid sequence at least 70% identical, more preferably at least 75% identical, and still more preferably 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a polypeptide of the present invention. Further included in the invention are isolated nucleic acid molecules encoding such polypeptides. Methods for determining identity include those well known in the art and described herein.

35 A further embodiment of the present invention is a method of making a protein comprising one of the sequences of SEQ ID NO: 74-123, comprising the steps of obtaining a cDNA comprising one of the sequences of sequence of SEQ ID NO: 24-73, inserting the cDNA in an expression vector

such that the cDNA is operably linked to a promoter, and introducing the expression vector into a host cell whereby the host cell produces the protein encoded by said cDNA. In one aspect of this embodiment, the method further comprises the step of isolating the protein.

Another embodiment of the present invention is a protein obtainable by the method  
5 described in the preceding paragraph.

Another embodiment of the present invention is a method of making a protein comprising the amino acid sequence of the mature protein contained in one of the sequences of SEQ ID NO: 74-123, comprising the steps of obtaining a cDNA comprising one of the nucleotides sequence of sequence of SEQ ID NO: 24-73 which encode for the mature protein, inserting the cDNA in an  
10 expression vector such that the cDNA is operably linked to a promoter, and introducing the expression vector into a host cell whereby the host cell produces the mature protein encoded by the cDNA. In one aspect of this embodiment, the method further comprises the step of isolating the protein.

Another embodiment of the present invention is a mature protein obtainable by the method  
15 described in the preceding paragraph.

Another embodiment of the present invention is a host cell containing the purified or isolated nucleic acids comprising the sequence of one of SEQ ID NOs: 24-73 or a sequence complementary thereto described herein.

Another embodiment of the present invention is a host cell containing the purified or  
20 isolated nucleic acids comprising the full coding sequences of one of SEQ ID NOs: 24-73, wherein the full coding sequence comprises the sequence encoding the signal peptide and the sequence encoding the mature protein described herein.

Another embodiment of the present invention is a host cell containing the purified or isolated nucleic acids comprising the nucleotides of one of SEQ ID NOs: 24-73 which encode a  
25 mature protein which are described herein.

Another embodiment of the present invention is a host cell containing the purified or isolated nucleic acids comprising the nucleotides of one of SEQ ID NOs: 24-73 which encode the signal peptide which are described herein.

Another embodiment of the present invention is a purified or isolated antibody capable of  
30 specifically binding to a protein comprising the sequence of one of SEQ ID NOs: 74-123. In one aspect of this embodiment, the antibody is capable of binding to a polypeptide comprising at least 6 consecutive amino acids, at least 8 consecutive amino acids, or at least 10 consecutive amino acids of the sequence of one of SEQ ID NOs: 74-123.

Another embodiment of the present invention is an array of cDNAs or fragments thereof of  
35 at least 15 nucleotides in length which includes at least one of the sequences of SEQ ID NOs: 24-73, or one of the sequences complementary to the sequences of SEQ ID NOs: 24-73, or a fragment thereof of at least 15 consecutive nucleotides. In one aspect of this embodiment, the array includes

at least two of the sequences of SEQ ID NOs: 24-73, the sequences complementary to the sequences of SEQ ID NOs: 24-73, or fragments thereof of at least 15 consecutive nucleotides. In another aspect of this embodiment, the array includes at least five of the sequences of SEQ ID NOs: 24-73, the sequences complementary to the sequences of SEQ ID NOs: 24-73, or fragments thereof of at least 15 consecutive nucleotides.

A further embodiment of the invention encompasses purified polynucleotides comprising an insert from a clone deposited in an ECACC deposit, which contains the sequences of SEQ ID NOs. 25-40 and 42-46, having an accession No. 99061735 and named SignalTag 15061999 or deposited in an ECACC deposit having an accession No. 98121805 and named SignalTag 166-191, which contains SEQ ID NOs.: 47-73, or a fragment of these nucleic acids comprising a contiguous span of at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 nucleotides of said insert. In one aspect of this embodiment, the purified polynucleotide is recombinant.

An additional embodiment of the invention encompasses purified polypeptides which comprise, consist of, or consist essentially of an amino acid sequence encoded by the insert from a clone deposited in an ECACC deposit, which contains the sequences of SEQ ID NOs. 25-40 and 42-46, having an accession No. 99061735 and named SignalTag 15061999 or deposited in an ECACC deposit having an accession No. 98121805 and named SignalTag 166-191, which contains SEQ ID NOs.: 47-73, as well as polypeptides which comprise a fragment of said amino acid sequence consisting of a signal peptide, a mature protein, or a contiguous span of at least 5, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 amino acids encoded by said insert.

An additional embodiment of the invention encompasses purified polypeptides which comprise a contiguous span of at least 5, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 amino acids of SEQ ID NOs: 74-123, wherein said contiguous span comprises at least one of the amino acid positions which was not shown to be identical to a public sequence in the instant application. Also encompassed by the invention are purified polynucleotides encoding said polypeptides.

Another embodiment of the present invention is a computer readable medium having stored thereon a sequence selected from the group consisting of a cDNA code of SEQ ID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123.

Another embodiment of the present invention is a computer system comprising a processor and a data storage device wherein the data storage device has stored thereon a sequence selected from the group consisting of a cDNA code of SEQ ID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123. In some embodiments the computer system further comprises a sequence comparer and a data storage device having reference sequences stored thereon. For example, the sequence comparer may comprise a computer program which indicates polymorphisms. In other aspects of the computer system, the system further comprises an identifier which identifies features in said sequence.

Another embodiment of the present invention is a method for comparing a first sequence to a reference sequence wherein the first sequence is selected from the group consisting of a cDNA code of SEQID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123 comprising the steps of reading the first sequence and the reference sequence through use of a computer program which  
5 compares sequences and determining differences between the first sequence and the reference sequence with the computer program. In some aspects of this embodiment, said step of determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.

Another aspect of the present invention is a method for determining the level of identity  
10 between a first sequence and a reference sequence, wherein the first sequence is selected from the group consisting of a cDNA code of SEQID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123, comprising the steps of reading the first sequence and the reference sequence through the use of a computer program which determines identity levels and determining identity between the first sequence and the reference sequence with the computer program.

Another embodiment of the present invention is a method for identifying a feature in a sequence selected from the group consisting of a cDNA code of SEQID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123 comprising the steps of reading the sequence through the use of a computer program which identifies features in sequences and identifying features in the sequence with said computer program. In one aspect of this embodiment, the computer program  
15 comprises a computer program which identifies open reading frames. In a further embodiment, the computer program comprises a program that identifies linear or structural motifs in a polypeptide sequence.

### **Brief Description of the Drawings**

Figure 1 is a table with all of the parameters that can be used for each step of cDNA  
25 analysis.

Figure 2 is an analysis of the 43 amino terminal amino acids of all human SwissProt proteins to determine the frequency of false positives and false negatives using the techniques for signal peptide identification described herein.

Figure 3 provides a diagram of a RT-PCR-based method to isolate cDNAs containing  
30 sequences adjacent to 5'ESTs used to obtain them

Figure 4 provides a schematic description of the promoters isolated and the way they are assembled with the corresponding 5' tags.

Figure 5 describes the transcription factor binding sites present in each of these promoters.

Figure 6 is a block diagram of an exemplary computer system.

Figure 7 is a flow diagram illustrating one embodiment of a process 200 for comparing a  
35 new nucleotide or protein sequence with a database of sequences in order to determine the identity levels between the new sequence and the sequences in the database.



Figure 8 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous.

Figure 9 is a flow diagram illustrating one embodiment of an identifier process 300 for detecting the presence of a feature in a sequence.

5

### **Brief Description of the Tables**

Table I provides structural features of each cDNAs of SEQ ID NOs: 24-73, i.e., the locations of the full coding sequences, the locations of the nucleotides which encode the signal peptides, the locations of nucleotides which encode the mature proteins generated by cleavage of the signal peptides, the locations of stop codons, the locations of the polyA signals and the locations of polyA sites.

10

Table II provides structural features for each polypeptide of SEQ ID NOs: 74-123, i.e; the locations of the full length polypeptide, the locations of the signal peptides, and the locations of the mature polypeptide created by cleaving the signal peptide from the full length polypeptide.

Table III lists the positions of preferred fragments, defined as fragments not sharing more than 90% identity with any public sequence over at least 30 nucleotides in length, for some cDNAs of SEQ ID NOs:74-123.

15

Table IVa provides the positions of fragments which are preferably included in the present invention while Table IVb provides the positions of fragments which are preferably excluded from the present invention. Tables IVa and IVb provides for the inclusion and exclusion of polynucleotides in addition to those described elsewhere in the specification and is therefore, not meant as limiting description.

20

Table V provides the applicant's internal designation number assigned to each sequence identification number and indicates whether the sequence is a nucleic acid sequence or a polypeptide sequence.

25

Table VI list the Genset's libraries of tissues and cell types examined that express the polynucleotides of the present invention.

Table VII relates to the bias in spatial distribution of the polynucleotide sequences of the present invention.

Table VIII relates to the spatial distribution of the polynucleotide sequences of the sequence listing using information from public databases.

30

Table IX lists known biologically structural and functional domains for the cDNA of the present invention.

Table X lists antigenic peaks of predicted antigenic epitopes for cDNAs or the present invention.

35

Table XI lists the putative chromosomal location of the polynucleotides of the present invention.

### **Detailed Description of the Preferred Embodiment**

#### **I. Obtaining cDNA libraries including the 5'Ends of their Corresponding mRNAs**

The cDNAs of the present invention may include the entire coding sequence of the protein encoded by the corresponding mRNA, including the authentic translation start site, the signal  
5 sequence, and the sequence encoding the mature protein remaining after cleavage of the signal peptide. Such cDNAs are referred to herein as "full length cDNAs." Alternatively, the cDNAs may include only the sequence encoding the mature protein remaining after cleavage of the signal peptide, or only the sequence encoding the signal peptide.

The methods explained therein can also be used to obtain cDNAs which encode less than the  
10 entire coding sequence of the secreted proteins encoded by the genes corresponding to the cDNAs. In some embodiments, the cDNAs isolated using these methods encode at least 5 amino acids of one of the proteins encoded by the sequences of SEQ ID NOs: 24-73. In further embodiments, the cDNAs encode at least 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 consecutive amino acids of the proteins encoded by the sequences of SEQ ID NOs: 24-73. In a preferred embodiment,  
15 the cDNAs encode a full length protein sequence, which includes the protein coding sequences of SEQ ID NOs: 24-73.

The cDNAs of the present invention were obtained from cDNA libraries derived from mRNAs having intact 5' ends as described in Examples 1 to 5 using either a chemical or enzymatic approach.

20

#### **EXAMPLE 1**

##### **Preparation of mRNA**

Total human RNAs or polyA<sup>+</sup> RNAs derived from different tissues were respectively purchased from LABIMO and CLONTECH and used to generate cDNA libraries as described below. The purchased RNA had been isolated from cells or tissues using acid guanidium  
25 thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, *Analytical Biochemistry* 162:156-159, 1987). PolyA<sup>+</sup> RNA was isolated from total RNA (LABIMO) by two passes of oligo dT chromatography, as described by Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412, 1972) in order to eliminate ribosomal RNA.

The quality and the integrity of the polyA<sup>+</sup> RNAs were checked. Northern blots hybridized  
30 with a probe corresponding to an ubiquitous mRNA, such as elongation factor 1 or elongation factor 2, were used to confirm that the mRNAs were not degraded. Contamination of the polyA<sup>+</sup> mRNAs by ribosomal sequences was checked using Northern blots and a probe derived from the sequence of the 28S rRNA. Preparations of mRNAs with less than 5% of rRNAs were used in library construction. To avoid constructing libraries with RNAs contaminated by exogenous sequences  
35 (prokaryotic or fungal), the presence of bacterial 16S ribosomal sequences or of two highly expressed fungal mRNAs was examined using PCR.

#### **EXAMPLE 2**

Methods for Obtaining mRNAs having Intact 5' Ends

Following preparation of the mRNAs from various tissues as described above, selection of mRNA with intact 5' ends and specific attachment of an oligonucleotide tag to the 5' end of such mRNA is performed using either a chemical or enzymatic approach. Both techniques take  
5 advantage of the presence of the "cap" structure, which characterizes the 5' end of intact mRNAs and which comprises a guanosine generally methylated once, at the 7 position.

The chemical modification approach involves the optional elimination of the 2', 3'-cis diol of the 3' terminal ribose, the oxidation of the 2', 3', -cis diol of the ribose linked to the cap of the 5' ends of the mRNAs into a dialdehyde, and the coupling of the dialdehyde to a derivatized  
10 oligonucleotide tag. Further detail regarding the chemical approaches for obtaining mRNAs having intact 5' ends are disclosed in International Application No. WO96/34981, published November 7, 1996, the disclosure of which is incorporated herein by reference in its entirety.

The enzymatic approach for ligating the oligonucleotide tag to the 5' ends of mRNAs with intact 5' ends involves the removal of the phosphate groups present on the 5' ends of uncapped  
15 incomplete mRNAs, the subsequent decapping of mRNAs with intact 5' ends and the ligation of the phosphate present at the 5' end of the decapped mRNA to an oligonucleotide tag. Further detail regarding the enzymatic approaches for obtaining mRNAs having intact 5' ends are disclosed in Dumas Milne Edwards J.B. (Doctoral Thesis of Paris VI University, Le clonage des ADNs complets: difficultes et perspectives nouvelles. Apports pour l'etude de la regulation de l'expression  
20 de la tryptophane hydroxylase de rat, 20 Dec. 1993), EP0 625572 and Kato *et al.*, *Gene* 150:243-250 (1994), the disclosures of which are incorporated herein by reference in their entireties.

In either the chemical or the enzymatic approach, the oligonucleotide tag has a restriction enzyme site (e.g. EcoRI sites) therein to facilitate later cloning procedures. Following attachment of the oligonucleotide tag to the mRNA, the integrity of the mRNA was then examined by performing a  
25 Northern blot using a probe complementary to the oligonucleotide tag.

**EXAMPLE 3**cDNA Synthesis Using mRNA Templates Having Intact 5' Ends

For the mRNAs joined to oligonucleotide tags using either the chemical or the enzymatic method, first strand cDNA synthesis was performed using reverse transcriptase with an oligo-dT  
30 primer or random nonamer. In some instances, this oligo-dT primer contained an internal tag of at least 4 nucleotides which is different from one tissue to the other. In order to protect internal EcoRI sites in the cDNA from digestion at later steps in the procedure, methylated dCTP was used for first strand synthesis. After removal of RNA by an alkaline hydrolysis, the first strand of cDNA was precipitated using isopropanol in order to eliminate residual primers.

35 The second strand of the cDNA was then synthesized with a Klenow fragment using a primer corresponding to the 5' end of the ligated oligonucleotide. Preferably, the primer is 20-25

bases in length. Methylated dCTP was also used for second strand synthesis in order to protect internal EcoRI sites in the cDNA from digestion during the cloning process.

#### EXAMPLE 4

##### Cloning of cDNAs derived from mRNA with intact 5' ends into BlueScript

5        Following second strand synthesis, the cDNAs were cloned into the phagemid pBlueScript II SK- vector (Stratagene). The ends of the cDNAs were blunted with T4 DNA polymerase (Biolabs) and the cDNA was digested with EcoRI. Since methylated dCTP was used during cDNA synthesis, the EcoRI site present in the tag was the only hemi-methylated site, hence the only site susceptible to EcoRI digestion. In some instances, to facilitate subcloning, an Hind III adaptor was added to the 3' end of cDNAs.

10        The cDNAs were then size fractionated using either exclusion chromatography (AcA, Biosepra) or electrophoretic separation which yields 3 or 6 different fractions. The cDNAs were then directionally cloned either into pBlueScript using either the EcoRI and SmaI restriction sites or the EcoRI and Hind III restriction sites when the Hind III adaptor was present in the cDNAs. The 15 ligation mixture was electroporated into bacteria and propagated under appropriate antibiotic selection.

#### EXAMPLE 5

##### Selection of Clones Having the Oligonucleotide Tag Attached Thereto

Clones containing the oligonucleotide tag attached to cDNAs were then selected as follows.

20        The plasmid DNAs containing cDNA libraries made as described above were purified (Qiagen). A positive selection of the tagged clones was performed as follows. Briefly, in this selection procedure, the plasmid DNA was converted to single stranded DNA using gene II endonuclease of the phage F1 in combination with an exonuclease (Chang *et al.*, *Gene* 127:95-8, 1993) such as exonuclease III or T7 gene 6 exonuclease. The resulting single stranded DNA was 25 then purified using paramagnetic beads as described by Fry *et al.*, *Biotechniques*, 13: 124-131, 1992. In this procedure, the single stranded DNA was hybridized with a biotinylated oligonucleotide having a sequence corresponding to the 3' end of the oligonucleotide tag described in example 2. Preferably, the primer has a length of 20-25 bases. Clones including a sequence complementary to the biotinylated oligonucleotide were captured by incubation with streptavidin coated magnetic 30 beads followed by magnetic selection. After capture of the positive clones, the plasmid DNA was released from the magnetic beads and converted into double stranded DNA using a DNA polymerase such as the ThermoSequenase obtained from Amersham Pharmacia Biotech. Alternatively, protocols such as the Gene Trapper kit (Gibco BRL) may be used. The double stranded DNA was then electroporated into bacteria. The percentage of positive clones having the 5' 35 tag oligonucleotide was estimated to typically rank between 90 and 98% using dot blot analysis.

Following electroporation, the libraries were ordered in 384-microtiter plates (MTP). A copy of the MTP was stored for future needs. Then the libraries were transferred into 96 MTP.

## II. Characterization of the 5' Ends of Clones

In order to sequence only cDNAs which contain the 5' ends of their corresponding mRNA, a first round of sequencing was performed on the 5' end of clones as described in example 6. In some instances, only a partial sequence of the clone, therein referred to as "5'EST" was obtained. In other instances, the complete sequence of the clone, herein referred to as a "cDNA" is obtained. A computer analysis was then performed on the 5' ESTs or cDNAs as described in Examples 7 and 8 in order to evaluate the quality of the cDNA libraries and in order to select clones containing sequences of interest among cDNAs which contain the 5' ends of their corresponding mRNA.

### **EXAMPLE 6**

#### Sequencing of The 5'End of cDNA Clones

The 5' ends of cloned cDNAs were then sequenced as follows. Plasmid inserts were first amplified by PCR on PE 9600 thermocyclers (Perkin-Elmer, Applied Biosystems Division, Foster City, CA) using standard SETA-A and SETA-B primers (Genset SA), AmpliTaqGold (Perkin-Elmer), dNTPs (Boehringer), buffer and cycling conditions as recommended by the Perkin-Elmer Corporation.

PCR products were then sequenced using automatic ABI Prism 377 sequencers (Perkin Elmer). Sequencing reactions were performed using PE 9600 thermocyclers with standard dye-primer chemistry and ThermoSequenase (Amersham Pharmacia Biotech). The primers used were either T7 or 21M13 (available from Genset SA) as appropriate. The primers were labeled with the JOE, FAM, ROX and TAMRA dyes. The dNTPs and ddNTPs used in the sequencing reactions were purchased from Boehringer. Sequencing buffer, reagent concentrations and cycling conditions were as recommended by Amersham.

Following the sequencing reaction, the samples were precipitated with ethanol, resuspended in formamide loading buffer, and loaded on a standard 4% acrylamide gel. Electrophoresis was performed for 2.5 hours at 3000V on an ABI 377 sequencer, and the sequence data were collected and analyzed using the ABI Prism DNA Sequencing Analysis Software, version 2.1.2.

The sequence data obtained from the sequencing of 5' ends of all cDNA libraries made as described above were transferred to a proprietary database, where quality control and validation steps were performed. A proprietary base-caller, working using a Unix system automatically flagged suspect peaks, taking into account the shape of the peaks, the inter-peak resolution, and the noise level. The proprietary base-caller also performed an automatic trimming. Any stretch of 25 or fewer bases having more than 4 suspect peaks was considered unreliable and was discarded. Sequences corresponding to cloning vector or ligation oligonucleotides were automatically removed from the sequences. However, the resulting sequences may contain 1 to 5 nucleotides belonging to the above mentioned sequences at their 5' end. If needed, these can easily be removed on a case by case basis.

Following sequencing as described above, the sequences of the cDNA clones were entered in a database for storage and manipulation as described below. Before searching the cDNA clones in the database for sequences of interest, cDNAs derived from mRNAs which were not of interest were identified and eliminated, namely, endogenous contaminants (ribosomal RNAs, transfer RNAs, mitochondrial RNAs) and exogenous contaminants (prokaryotic RNAs and fungal RNAs) using software and parameters described in Figure 1. In addition, cDNA sequences showing identity to repeated sequences (Alu, L1, THE and MER repeats, SSTR sequences or satellite, micro-satellite, or telomeric repeats) were identified and masked in further processing.

#### EXAMPLE 7

##### 10 Determination of Efficiency of 5' End Selection

To determine the efficiency at which the above selection procedures isolated cDNAs which include the 5' ends of their corresponding mRNAs, the sequences of 5'ESTs or cDNAs were aligned with a reference pool of complete mRNA/cDNA extracted from the EMBL release 57 using the FASTA algorithm. The reference mRNA/cDNA starting at the most 5' transcription start site was 15 obtained, and then compared to the 5' transcription start site position of the 5'EST or cDNA. More than 75% of 5'ESTs or cDNAs had their 5' ends close to the 5' ends of the known sequence. As some of the mRNA sequences available in the EMBL database are deduced from genomic sequences, a 5' end matching with these sequences will be counted as an internal match. Thus, the method used here underestimates the yield of 5'ESTs or cDNAs including the authentic 5' ends of 20 their corresponding mRNAs.

#### EXAMPLE 8

##### Identification of Open Reading Frames Coding For Potential Signal Peptides

The obtained nucleic acid sequences were then screened to identify those having uninterrupted open reading frames (ORF) with a good coding probability using proprietary software. 25 When the full-length cDNA was obtained, only complete ORFs, namely nucleic acid sequences beginning with a start codon and ending with a stop codon, longer than 150 nucleotides were considered. When only 5'EST sequences were obtained, both complete ORFs longer than 150 nucleotides and incomplete ORFs, namely nucleic acid sequences beginning with a start codon and extending up to the end of the 5'EST, longer than 60 nucleotides were considered.

30 The retrieved ORFs were then searched to identify potential signal motifs using slight modifications of the procedures disclosed in Von Heijne, *Nucleic Acids Res.* 14:4683-4690, 1986, the disclosure of which is incorporated herein by reference. Those 5'ESTs or cDNA sequences encoding a polypeptide with a score of at least 3.5 in the Von Heijne signal peptide identification matrix were considered to possess a signal sequence. Those 5'ESTs or cDNAs which matched a 35 known human mRNA or EST sequence and had a 5' end more than 30 nucleotides downstream of the known 5' end were excluded from further analysis.

#### EXAMPLE 9

Confirmation of Accuracy of Identification of Potential Signal Sequences in 5' ESTs

The accuracy of the above procedure for identifying signal sequences encoding signal peptides was evaluated by applying the method to the 43 amino acids located at the N terminus of all human SwissProt proteins. The computed Von Heijne score for each protein was compared with the known characterization of the protein as being a secreted protein or a non-secreted protein. In this manner, the number of non-secreted proteins having a score higher than 3.5 (false positives) and the number of secreted proteins having a score lower than 3.5 (false negatives) could be calculated.

Using the results of the above analysis, the probability that a peptide encoded by the 5' region of the mRNA is in fact a genuine signal peptide based on its Von Heijne's score was calculated based on either the assumption that 10% of human proteins are secreted or the assumption that 20% of human proteins are secreted. The results of this analysis are shown in figure 2.

Using the above method of identification of secretory proteins, 5' ESTs of the following polypeptides known to be secreted were obtained: human glucagon, gamma interferon induced monokine precursor, secreted cyclophilin-like protein, human pleiotropin, and human biotinidase precursor. Thus, the above method successfully identified those 5' ESTs which encode a signal peptide.

To confirm that the signal peptide encoded by the 5' ESTs or cDNAs actually functions as a signal peptide, the signal sequences from the 5' ESTs or cDNAs may be cloned into a vector designed for the identification of signal peptides. Such vectors are designed to confer the ability to grow in selective medium only to host cells containing a vector with an operably linked signal sequence. For example, to confirm that a 5' EST or cDNA encodes a genuine signal peptide, the signal sequence of the 5' EST or cDNA may be inserted upstream and in frame with a non-secreted form of the yeast invertase gene in signal peptide selection vectors such as those described in U.S. Patent No. 5,536,637, the disclosure of which is incorporated herein by reference. Growth of host cells containing signal sequence selection vectors with the correctly inserted 5' EST or cDNA signal sequence confirms that the 5' EST or cDNA encodes a genuine signal peptide.

Alternatively, the presence of a signal peptide may be confirmed by cloning the 5'ESTs or cDNAs into expression vectors such as pXT1 as described below, or by constructing promoter-signal sequence-reporter gene vectors which encode fusion proteins between the signal peptide and an assayable reporter protein. After introduction of these vectors into a suitable host cell, such as COS cells or NIH 3T3 cells, the growth medium may be harvested and analyzed for the presence of the secreted protein. The medium from these cells is compared to the medium from control cells containing vectors lacking the signal sequence or cDNA insert to identify vectors which encode a functional signal peptide or an authentic secreted protein.

Evaluation of Expression Levels and Patterns of mRNAs Corresponding to 5' ESTs or cDNAs

The spatial and temporal expression patterns of the mRNAs corresponding to the 5' ESTs or cDNAs, as well as their expression levels, may be determined. Characterization of the spatial and temporal expression patterns and expression levels of these mRNAs is useful for constructing  
5 expression vectors capable of producing a desired level of gene product in a desired spatial or temporal manner, as will be discussed in more detail below.

In addition, cDNAs or 5' ESTs whose corresponding mRNAs are associated with disease states may also be identified. For example, a particular disease may result from lack of expression, over expression, or under expression of an mRNA corresponding to a cDNA or 5' EST. By  
10 comparing mRNA expression patterns and quantities in samples taken from healthy individuals with those from individuals suffering from a particular disease, cDNAs and 5' ESTs responsible for the disease may be identified.

Expression levels and patterns of mRNAs corresponding to 5' ESTs or cDNAs may be analyzed by solution hybridization with long probes as described in International Patent Application  
15 No. WO 97/05277, the entire contents of which are hereby incorporated by reference. Briefly, a 5' EST, cDNA, or fragment thereof corresponding to the gene encoding the mRNA to be characterized is inserted at a cloning site immediately downstream of a bacteriophage (T3, T7 or SP6) RNA polymerase promoter to produce antisense RNA. Preferably, the 5' EST or cDNA is 100 or more nucleotides in length. The plasmid is linearized and transcribed in the presence of ribonucleotides  
20 comprising modified ribonucleotides (i.e. biotin-UTP and DIG-UTP). An excess of this doubly labeled RNA is hybridized in solution with mRNA isolated from cells or tissues of interest. The hybridizations are performed under standard stringent conditions (40-50°C for 16 hours in an 80% formamide, 0.4 M NaCl buffer, pH 7-8). The unhybridized probe is removed by digestion with ribonucleases specific for single-stranded RNA (i.e. RNases CL3, T1, Phy M, U2 or A). The  
25 presence of the biotin-UTP modification enables capture of the hybrid on a microtitration plate coated with streptavidin. The presence of the DIG modification enables the hybrid to be detected and quantified by ELISA using an anti-DIG antibody coupled to alkaline phosphatase.

The 5' ESTs, cDNAs, or fragments thereof may also be tagged with nucleotide sequences for the serial analysis of gene expression (SAGE) as disclosed in UK Patent Application No. 2 305  
30 241 A, the entire contents of which are incorporated by reference. In this method, cDNAs are prepared from a cell, tissue, organism or other source of nucleic acid for which it is desired to determine gene expression patterns. The resulting cDNAs are separated into two pools. The cDNAs in each pool are cleaved with a first restriction endonuclease, called an "anchoring enzyme," having a recognition site which is likely to be present at least once in most cDNAs. The fragments which  
35 contain the 5' or 3' most region of the cleaved cDNA are isolated by binding to a capture medium such as streptavidin coated beads. A first oligonucleotide linker having a first sequence for hybridization of an amplification primer and an internal restriction site for a "tagging endonuclease"



is ligated to the digested cDNAs in the first pool. Digestion with the second endonuclease produces short "tag" fragments from the cDNAs.

A second oligonucleotide having a second sequence for hybridization of an amplification primer and an internal restriction site is ligated to the digested cDNAs in the second pool. The  
5 cDNA fragments in the second pool are also digested with the "tagging endonuclease" to generate short "tag" fragments derived from the cDNAs in the second pool. The "tags" resulting from digestion of the first and second pools with the anchoring enzyme and the tagging endonuclease are ligated to one another to produce "ditags." In some embodiments, the ditags are concatamerized to produce ligation products containing from 2 to 200 ditags. The tag sequences are then determined  
10 and compared to the sequences of the 5' ESTs or cDNAs to determine which 5' ESTs or cDNAs are expressed in the cell, tissue, organism, or other source of nucleic acids from which the tags were derived. In this way, the expression pattern of the 5' ESTs or cDNAs in the cell, tissue, organism, or other source of nucleic acids is obtained.

Quantitative analysis of gene expression may also be performed using arrays. As used  
15 herein, the term array means a one dimensional, two dimensional, or multidimensional arrangement of full length cDNAs (i.e. cDNAs which include the coding sequence for the signal peptide, the coding sequence for the mature protein, and a stop codon), cDNAs, 5' ESTs or fragments of the full length cDNAs, cDNAs, or 5' ESTs of sufficient length to permit specific detection of gene expression. Preferably, the fragments are at least 15 nucleotides in length. More preferably, the  
20 fragments are at least 100 nucleotides in length. More preferably, the fragments are more than 100 nucleotides in length. In some embodiments the fragments may be more than 500 nucleotides in length.

For example, quantitative analysis of gene expression may be performed with full length cDNAs, cDNAs, 5' ESTs, or fragments thereof in a complementary DNA microarray as described by  
25 Schena *et al.* (*Science* 270:467-470, 1995; *Proc. Natl. Acad. Sci. U.S.A.* 93:10614-10619, 1996). Full length cDNAs, cDNAs, 5' ESTs or fragments thereof are amplified by PCR and arrayed from 96-well microtiter plates onto silylated microscope slides using high-speed robotics. Printed arrays are incubated in a humid chamber to allow rehydration of the array elements and rinsed, once in 0.2% SDS for 1 min, twice in water for 1 min and once for 5 min in sodium borohydride solution.  
30 The arrays are submerged in water for 2 min at 95°C, transferred into 0.2% SDS for 1 min, rinsed twice with water, air dried and stored in the dark at 25°C.

Cell or tissue mRNA is isolated or commercially obtained and probes are prepared by a single round of reverse transcription. Probes are hybridized to 1 cm<sup>2</sup> microarrays under a 14 x 14 mm glass coverslip for 6-12 hours at 60°C. Arrays are washed for 5 min at 25°C in low stringency  
35 wash buffer (1 x SSC/0.2% SDS), then for 10 min at room temperature in high stringency wash buffer (0.1 x SSC/0.2% SDS). Arrays are scanned in 0.1 x SSC using a fluorescence laser scanning

device fitted with a custom filter set. Accurate differential expression measurements are obtained by taking the average of the ratios of two independent hybridizations.

Quantitative analysis of the expression of genes may also be performed with full length cDNAs, cDNAs, 5' ESTs, or fragments thereof in complementary DNA arrays as described by Pietu  
5 *et al.* (Genome Research 6:492-503, 1996). The full length cDNAs, cDNAs, 5' ESTs or fragments thereof are PCR amplified and spotted on membranes. Then, mRNAs originating from various tissues or cells are labeled with radioactive nucleotides. After hybridization and washing in controlled conditions, the hybridized mRNAs are detected by phospho-imaging or autoradiography. Duplicate experiments are performed and a quantitative analysis of differentially expressed mRNAs  
10 is then performed.

Alternatively, expression analysis of the 5' ESTs or cDNAs can be done through high density nucleotide arrays as described by Lockhart *et al.* (Nature Biotechnology 14: 1675-1680, 1996) and Sosnowsky *et al.* (Proc. Natl. Acad. Sci. 94:1119-1123, 1997). Oligonucleotides of 15-50 nucleotides corresponding to sequences of the 5' ESTs or cDNAs are synthesized directly on the  
15 chip (Lockhart *et al.*, *supra*) or synthesized and then addressed to the chip (Sosnowski *et al.*, *supra*). Preferably, the oligonucleotides are about 20 nucleotides in length.

cDNA probes labeled with an appropriate compound, such as biotin, digoxigenin or fluorescent dye, are synthesized from the appropriate mRNA population and then randomly fragmented to an average size of 50 to 100 nucleotides. The said probes are then hybridized to the  
20 chip. After washing as described in Lockhart *et al.*, *supra* and application of different electric fields (Sosnowsky *et al.*, Proc. Natl. Acad. Sci. 94:1119-1123), the dyes or labeling compounds are detected and quantified. Duplicate hybridizations are performed. Comparative analysis of the intensity of the signal originating from cDNA probes on the same target oligonucleotide in different cDNA samples indicates a differential expression of the mRNA corresponding to the 5' EST or  
25 cDNA from which the oligonucleotide sequence has been designed.

### III. Characterization of cDNAs including the 5' End of their Corresponding mRNA

#### **EXAMPLE 11**

##### Characterization of the complete sequence of cDNA clones

Clones which include the 5' end of their corresponding mRNA and which encode a new  
30 protein with a signal peptide as determined in the aforementioned procedure were then fully sequenced as follows.

First, both 5' and 3' ends of cloned cDNAs were sequenced twice in order to confirm the identity of the clone using a Die Terminator approach with the AmpliTaq DNA polymerase FS kit available from Perkin Elmer. Second, primer walking was performed if the full coding region had  
35 not been obtained yet using software such as OSP to choose primers and automated computer software such as ASMG (Sutton *et al.*, *Genome Science Technol.* 1: 9-19, 1995) to construct contigs of walking sequences including the initial 5' tag. Contiguation was then performed using 5' and 3'

sequences and eventually primer walking sequences. The sequence was considered complete when the resulting contigs included the full coding region as well as overlapping sequences with vector DNA on both ends. In addition, clones were entirely sequenced in order to obtain at least two sequences per clone. Preferably, the sequences were obtained from both sense and antisense strands.

- 5 All the contigated sequences for each clone were then used to obtain a consensus sequence which was then submitted to the computer analysis described below.

Alternatively, clones which include the 5' end of their corresponding mRNA and which encode a new protein with a signal peptide, as determined in the aforementioned procedure, may be subcloned into an appropriate vector such as pED6dpc2 (DiscoverEase, Genetics Institute,  
10 Cambridge, MA) before full sequencing.

### EXAMPLE 12

#### Determination of Structural and Functional Features

Following identification of contaminants and masking of repeats, structural features, e.g. polyA tail and polyadenylation signal, of the sequences of cDNAs were subsequently determined  
15 using the algorithm, parameters and criteria defined in figure 1. Briefly, a polyA tail was defined as a homopolymeric stretch of at least 11 A with at most one alternative base within it. The polyA tail search was restricted to the last 100 nt of the sequence and limited to stretches of 11 consecutive A's because sequencing reactions are often not readable after such a polyA stretch. To search for a polyadenylation signal, the polyA tail was clipped from the full-length sequence. The 50 bp  
20 preceding the polyA tail were searched for the canonic polyadenylation AAUAAA signal allowing one mismatch to account for possible sequencing errors as well as known variation in the canonical sequence of the polyadenylation signal.

Functional features, e.g. ORFs and signal sequences, of the sequences of cDNAs were subsequently determined as follows. The 3 upper strand frames of cDNAs were searched for ORFs  
25 defined as the maximum length fragments beginning with a translation initiation codon and ending with a stop codon. ORFs encoding at least 80 amino acids were preferred. Each found ORF was then scanned for the presence of a signal peptide using the matrix method described in example 10.

Sequences of cDNAs were then compared, on a nucleotidic or proteic basis, to public sequences available at the time of filing.

30

### EXAMPLE 13

#### Selection of Full Length Sequences

cDNAs that had already been characterized by the aforementioned computer analysis were then submitted to an automatic procedure in order to preselect cDNAs containing sequences of interest.

- 35 a) *Automatic sequence preselection*

All cDNAs clipped for vector on both ends were considered. First, a negative selection was performed in order to eliminate sequences which resulted from either contaminants or artifacts as

follows. Sequences matching contaminant sequences were discarded as well as those encoding ORF sequences exhibiting identity to repeats. Sequences lacking polyA tail were also discarded. Those cDNAs which matched a known human mRNA or EST sequence and had a 5' end more than 30 nucleotides downstream of the known 5' end were also excluded from further analysis. Only ORFs  
5 ending before the polyA tail were kept.

Then, for each remaining cDNA containing several ORFs, a preselection of ORFs was performed using the following criteria. The longest ORF was preferred. If the ORF sizes were similar, the chosen ORF was the one which signal peptide had the highest score according to Von Heijne method as defined in Example 10.

10 Sequences of cDNA clones were then compared pairwise with BLAST after masking of the repeat sequences. Sequences containing at least 90% identity over 30 nucleotides were clustered in the same class. Each cluster was then subjected to a clustal analysis that detects sequences resulting from internal priming or from alternative splicing, identical sequences or sequences with several frameshifts. This automatic analysis served as a basis for manual selection of the sequences.

15 *b) Manual sequence selection*

Manual selection was carried out using automatically generated reports for each sequenced cDNA clone. During the manual selection procedure, a selection was performed between clones belonging to the same class as follows. ORF sequences encoded by clones belonging to the same class were aligned and compared. If the identity between nucleotidic sequences of clones belonging  
20 to the same class was more than 90% over 30 nucleotide stretches or if the identity between amino acid sequences of clones belonging to the same class was more than 80% over 20 amino acid stretches, then the clones were considered as being identical. The chosen ORF was either the one exhibiting matches with known amino acid sequences or the best one according to the criteria mentioned in the automatic sequence preselection section. If the nucleotide and amino acid  
25 homologies were less than 90% and 80% respectively, the clones were said to encode distinct proteins which can be both selected if they contain sequences of interest.

Selection of full length cDNA clones encoding sequences of interest was performed using the following criteria. Structural parameters (initial tag, polyadenylation site and signal, eventually matches with public ESTs in 5' or 3' of the sequence) were first checked in order to confirm that the  
30 cDNA was complete in 5' and in 3'. Then, homologies with known nucleic acids and proteins were examined in order to determine whether the clone sequence matched a known nucleic acid or protein sequence and, in the latter case, its covering rate and the date at which the sequence became public. If there was no extensive match with sequences other than ESTs or genomic DNA, or if the clone sequence included substantial new information, such as encoding a protein resulting from alternative  
35 splicing of an mRNA coding for an already known protein, the sequence was kept. Examples of such cloned full length cDNAs containing sequences of interest are described in Example 14.

Sequences resulting from chimera or double inserts as assessed by identity to other sequences were discarded during this procedure.

#### EXAMPLE 14

##### Characterization of Full-length cDNAs

5       The procedure described above was used to obtain or full length cDNAs derived from a variety of tissues. The following list provides a few examples of thus obtained cDNAs.

Using this procedure, the full length cDNA of SEQ ID NO:1 (internal identification number 108-005-5-0-F9-FLC) was obtained. This cDNA encodes a potentially secreted protein (SEQ ID NO:2) with a signal peptide having a von Heijne score of 4.1.

10       Using this procedure, the full length cDNA of SEQ ID NO:3 (internal identification number 108-004-5-0-G10-FLC) was obtained. This cDNA encodes a potentially secreted protein (SEQ ID NO:4) with a signal peptide having a von Heijne score of 5.3.

Using this procedure, the full length cDNA of SEQ ID NO:5 (internal identification number 108-004-5-0-B12-FLC) was obtained. This cDNA encodes a potentially secreted protein (SEQ ID  
15 NO:6) with a signal peptide having a von Heijne score of 7.0.

Using this procedure, the full length cDNA of SEQ ID NO:7 (internal identification number 108-013-5-0-G5-FLC) was obtained. This cDNA encodes a potentially secreted protein (SEQ ID NO:8) with a signal peptide having a von Heijne score of 9.4.

Furthermore, the polypeptides encoded by the extended or full-length cDNAs may be  
20 screened for the presence of known structural or functional motifs or for the presence of signatures, small amino acid sequences which are well conserved amongst the members of a protein family. Some of the results obtained for the polypeptides encoded by full-length cDNAs that were screened for the presence of known protein signatures and motifs using the Proscan software from the GCG package and the Prosite database are provided below.

25       The protein of SEQ ID NO :10 encoded by the full-length cDNA SEQ ID NO:9 (internal designation 108-013-5-0-H9-FLC) shows homologies with a family of lysophospholipases conserved among eukaryotes (yeast, rabbit, rodents and human). In addition, some members of this family exhibit a calcium-independent phospholipase A2 activity (Portilla *et al.*, *J. Am. Soc. Nephro.*, 9 :1178-1186 (1998)). All members of this family exhibit the active site consensus GX SXG motif  
30 of carboxylesterases that is also found in the protein of SEQ ID NO :10 (position 54 to 58). In addition, this protein may be a membrane protein with one transmembrane domain as predicted by the software TopPred II (Claros and von Heijne, *CABIOS applic. Notes*, 10 :685-686 (1994)). Taken together, these data suggest that the protein of SEQ ID NO:10 may play a role in fatty acid  
35 diagnosing and/or treating several disorders including, but not limited to, cancer, diabetes, and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. It may also be useful in modulating inflammatory responses to infectious agents and/or to suppress graft rejection.

The protein of SEQ ID NO: 12 encoded by the full-length cDNA SEQ ID NO:11 (internal designation 108-004-5-0-D10-FLC) shows remote identity to a subfamily of beta4-galactosyltransferases widely conserved in animals (human, rodents, cow and chicken). Such enzymes, usually type II membrane proteins located in the endoplasmic reticulum or in the Golgi apparatus, catalyze the biosynthesis of glycoproteins, glycolipid glycans and lactose. Their characteristic features defined as those of subfamily A in Breton *et al.*, *J. Biochem.*, 123:1000-1009 (1998) are pretty well conserved in the protein of SEQ ID NO: 12, especially the region I containing the DVD motif (positions 163-165) thought to be involved either in UDP binding or in the catalytic process itself. In addition, the protein of SEQ ID NO: 12 has the typical structure of a type II protein. Indeed, it contains a short 28-amino-acid-long N-terminal tail, a transmembrane segment from positions 29 to 49 and a large 278-amino-acid-long C-terminal tail as predicted by the software TopPred II (Claros and von Heijne, *CABIOS applic. Notes*, 10 :685-686 (1994)). Taken together, these data suggest that the protein of SEQ ID NO: 12 may play a role in the biosynthesis of polysaccharides, and of the carbohydrate moieties of glycoproteins and glycolipids and/or in cell-cell recognition. Thus, this protein may be useful in diagnosing and/or treating several types of disorders including, but not limited to, cancer, atherosclerosis, cardiovascular disorders, autoimmune disorders and rheumatic diseases including rheumatoid arthritis.

The protein of SEQ ID NO: 14 encoded by the extended cDNA SEQ ID NO: 13 (internal designation 108-004-5-0-E8-FLC) exhibits the typical PROSITE signature for amino acid permeases (positions 5 to 66) which are integral membrane proteins involved in the transport of amino acids into the cell. In addition, the protein of SEQ ID NO: 14 has a transmembrane segment from positions 9 to 29 as predicted by the software TopPred II (Claros and von Heijne, *CABIOS applic. Notes*, 10 :685-686 (1994)). Taken together, these data suggest that the protein of SEQ ID NO: 14 may be involved in amino acid transport. Thus, this protein may be useful in diagnosing and/or treating several types of disorders including, but not limited to, cancer, aminoacidurias, neurodegenerative diseases, anorexia, chronic fatigue, coronary vascular disease, diphtheria, hypoglycemia, male infertility, muscular and myopathies.

Bacterial clones containing plasmids containing the full length cDNAs described above are presently stored in the inventor's laboratories under the internal identification numbers provided above. The inserts may be recovered from the deposited materials by growing an aliquot of the appropriate bacterial clone in the appropriate medium. The plasmid DNA can then be isolated using plasmid isolation procedures familiar to those skilled in the art such as alkaline lysis minipreps or large scale alkaline lysis plasmid isolation procedures. If desired the plasmid DNA may be further enriched by centrifugation on a cesium chloride gradient, size exclusion chromatography, or anion exchange chromatography. The plasmid DNA obtained using these procedures may then be manipulated using standard cloning techniques familiar to those skilled in the art. Alternatively, a PCR can be done with primers designed at both ends of the cDNA insertion. The PCR product

which corresponds to the cDNA can then be manipulated using standard cloning techniques familiar to those skilled in the art.

The above procedure was also used to obtain the cDNAs of the invention comprising the sequences of SEQ ID NOs: 24-73. Table I provides the sequence identification numbers of the cDNAs of the present invention, the locations of the first and last nucleotides of the full coding sequences in SEQ ID NOs: 24-73 (i.e. the nucleotides encoding both the signal peptide and the mature protein, listed under the heading FCS location in Table I), the locations of the first and last nucleotides in SEQ ID NOs: 24-73 which encode the signal peptides (listed under the heading SigPep Location in Table I), the locations of the first and last nucleotides in SEQ ID NOs: 24-73 which encode the mature proteins generated by cleavage of the signal peptides (listed under the heading Mature Polypeptide Location in Table I), the locations in SEQ ID NOs: 24-73 of stop codons (listed under the heading Stop Codon Location in Table I), the locations of the first and last nucleotides in SEQ ID NOs: 24-73 of the polyA signals (listed under the heading Poly A Signal Location in Table I) and the locations of the first and last nucleotides of the polyA sites (listed under the heading Poly A Site Location in Table I).

Table II lists the sequence identification numbers of the polypeptides of SEQ ID NOs: 74-123, the locations of the first and last amino acid residues of SEQ ID NOs: 74-123 in the full length polypeptide (second column), the locations of the first and last amino acid residues of SEQ ID NOs: 74-123 in the signal peptides (third column), and the locations of the first and last amino acid residues of SEQ ID NOs: 74-123 in the mature polypeptide created by cleaving the signal peptide from the full length polypeptide (fourth column).

The nucleotide sequences of the sequences of SEQ ID NOs: 24-73 and the amino acid sequences encoded by SEQ ID NOs: 24-73 (i.e. amino acid sequences of SEQ ID NOs: 74-123) are provided in the appended sequence listing. In some instances, the sequences are preliminary and may include some incorrect or ambiguous sequences or amino acids. All instances of the symbol "n" in the nucleic acid sequences mean that the nucleotide can be adenine, guanine, cytosine or thymine. For each amino acid sequence, Applicants have identified what they have determined to be the reading frame best identifiable with sequence information available at the time of filing. In some instances the polypeptide sequences in the Sequence Listing contain the symbol "Xaa." These "Xaa" symbols indicate either (1) a residue which cannot be identified because of nucleotide sequence ambiguity or (2) a stop codon in the determined sequence where applicants believe one should not exist (if the sequence were determined more accurately). Thus, "Xaa" indicates that a residue may be any of the twenty amino acids. In some instances, several possible identities of the unknown amino acids may be suggested by the genetic code.

The sequences of SEQ ID NOs: 24-73 can readily be screened for any errors therein and any sequence ambiguities can be resolved by resequencing a fragment containing such errors or ambiguities on both strands. Nucleic acid fragments for resolving sequencing errors or ambiguities

may be obtained from the deposited clones or can be isolated using the techniques described herein. Resolution of any such ambiguities or errors may be facilitated by using primers which hybridize to sequences located close to the ambiguous or erroneous sequences. For example, the primers may hybridize to sequences within 50-75 bases of the ambiguity or error. Upon resolution of an error or ambiguity, the corresponding corrections can be made in the protein sequences encoded by the DNA containing the error or ambiguity. The amino acid sequence of the protein encoded by a particular clone can also be determined by expression of the clone in a suitable host cell, collecting the protein, and determining its sequence.

#### EXAMPLE 15A

##### 10 Categorization of cDNAs of the Present Invention

The nucleic acid sequences of the present invention (SEQ ID NOs. 24-73) were grouped based on their identity to known sequences as follows. All sequences were compared to public sequences available at the time of filing the priority applications.

In some instances, the cDNAs did not match any known vertebrate sequence nor any publicly available EST sequence, thus being completely new.

All sequences exhibiting more than 90% of identity to known sequences over at least 30 nucleotides were retrieved and further analyzed. For these cDNAs referred to by their sequence identification numbers (first column), Table III gives the positions of preferred fragments within these sequences (second column entitled "Positions of preferred fragments"). Each fragment is represented by x-y where x and y are the start and end positions respectively of a given preferred fragment. Preferred fragments are separated from each other by a comma. As used herein the term "polynucleotide described in Table III" refers to the all of the preferred polynucleotide fragments defined in Table III in this manner.

In addition, Table IVa provides for preferred fragments of the polynucleotides of the invention while Table IVb provides for

For each polynucleotide referred to by its sequence identification number (first column), the second column of Table IVa provides the positions of fragments which are preferably included in the present invention (column 2) while the second column of IVb provides the positions of fragments which are preferably excluded from the present invention. Each fragment is represented by x-y where x and y are the start and end positions respectively of a given fragment. Fragments are separated from each other by a semi-column. Tables IVa and IVb provides for the inclusion and exclusion of polynucleotides in addition to those described elsewhere in the specification and is therefore, not meant as limiting description. As used herein the terms "polynucleotide described in Table IVa" and "polynucleotide described in Table IVb" refers to the all of the polynucleotide fragments defined in the second column of Tables IVa or IVb respectively in this manner.

The present invention encompasses isolated, purified, or recombinant nucleic acids which consist of, consist essentially of, or comprise a contiguous span of one of the sequences of SEQ ID Nos.



24-73 or a sequence complementary thereto, said contiguous span comprising at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 nucleotides of the sequence of SEQ ID Nos. 24-73 or a sequence complementary thereto, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular sequence, wherein  
5 the contiguous span comprises at least 1, 2, 3, 5, 10, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400 or 500 of a polynucleotide described in Table III or of a polynucleotide described in Table IVa, or a sequence complementary thereto. The present invention also encompasses isolated, purified, or recombinant nucleic acids comprising, consisting essentially of, or consisting of a contiguous span of at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400,  
10 500, 1000 or 2000 nucleotides of a polynucleotide described in Table III or of a polynucleotide described in Table IVa or a sequence complementary thereto, to the extent that a contiguous span of these lengths is consistent with the length of the particular sequence described in Table III. The present invention also encompasses isolated, purified, or recombinant nucleic acids which comprise, consist of or consist essentially of a polynucleotide described in Table III or of a polynucleotide described in Table  
15 IVa, or a sequence complementary thereto. The present invention further encompasses any combination of the nucleic acids listed in this paragraph.

Cells containing the cDNAs (SEQ ID NOs: 24-73) of the present invention in the vector pBluescriptII SK- (Stratagene) are maintained in permanent deposit by the inventors at Genset, S.A., 24 Rue Royale, 75008 Paris, France.

20 Pool of cells containing the cDNAs of SEQ ID NOs: 24-73, from which the cells containing a particular polynucleotide is obtainable, were deposited with the European Collection of Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom. Each cDNA clone has been transfected into separate bacterial cells (E-coli)  
25 for these composite deposits. In particular, cells containing the sequences of SEQ ID NOs: 25-40 and 42-46 were deposited on June, 17, 1999 in the pool having ECACC Accession No. 99061735 and designated SignalTag 15061999. In addition, cells containing the sequences of SEQ ID Nos: 47-73 were deposited on December 18, 1998, in the pool having ECACC Accession No. 98121805 and designated SignalTag 166-191. Table IV provides the internal designation number assigned to each  
30 SEQ ID NO. and indicates whether the sequence is a nucleic acid sequence or a protein sequence.

Each cDNA can be removed from the Bluescript vector in which it was deposited by performing a Bsh II double digestion to produce the appropriate fragment for each clone provided the cDNA clone sequence does not contain this restriction site. Alternatively, other restriction enzymes of the multicloning site of the vector may be used to recover the desired insert as indicated  
35 by the manufacturer.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences. The design of the oligonucleotide probe should preferably follow these parameters:

5 (a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;

(b) Preferably, the probe is designed to have a  $T_m$  of approx. 80°C (assuming 2 degrees for each A or T and 4 degrees for each G or C). However, probes having melting temperatures between 40 °C and 80 °C may also be used provided that specificity is not lost.

10 The oligonucleotide should preferably be labeled with (-[<sup>32</sup>P]ATP (specific activity 6000 Ci/mmol) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantified by measurement in a scintillation  
15 counter. Preferably, specific activity of the resulting probe should be approximately  $4 \times 10^6$  dpm/pmol.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 µg/ml. The culture should preferably be grown to saturation at 37°C,  
20 and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 µg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

25 Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 µg/ml of yeast RNA, and 10 mM EDTA (approximately 10 ml per 150 mm filter).  
30 Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to  $1 \times 10^6$  dpm/ml. The filter is then preferably incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 ml of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to  
35 visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

The plasmid DNA obtained using these procedures may then be manipulated using standard cloning techniques familiar to those skilled in the art. Alternatively, a PCR can be done with primers designed at both ends of the cDNA insertion. The PCR product which corresponds to the cDNA can then be manipulated using standard cloning techniques familiar to those skilled in the art.

Tissue expression of the cDNAs of the present invention was also examined. Table VI list the Genset's libraries of tissues and cell types examined that express the polynucleotides of the present invention. The tissues and cell types examined for polynucleotide expression were: brain, fetal brain, fetal kidney, fetal liver, pituitary gland, liver, placenta, prostate, salivary gland, stomach/intestine, and testis. For each cDNA referred to by its sequence identification number (first column), the number of proprietary 5'ESTs expressed in a particular tissue referred to by its name is indicated in parentheses (second column). In addition, the bias in the spatial distribution of the polynucleotide sequences of the present invention is indicated in Table VII. The expression of these sequences were examined by comparing the relative proportions of the biological polynucleotides of a given tissue using the following statistical analysis. The under- or over-representation of a polynucleotide of a given cluster in a given tissue was performed using the normal approximation of the binomial distribution. When the observed proportion of a polynucleotide of a given tissue in a given consensus had less than 1% chance to occur randomly according to the chi2 test, the frequency bias was reported as "preferred". The results are given in Table VII as follows. For each polynucleotide showing a bias in tissue distribution as referred to by its sequence identification number in the first column, the list of tissues where the polynucleotides are over-represented is given in the second column entitled "preferential expression".

In addition, the spatial distribution of the polynucleotide sequences of the present invention was investigated using information from public databases. The expression of the sequences of SEQ ID NOs:24-73 was examined by comparing them to the polynucleotide sequences in public databases. Table VIII lists tissues and cell types which express the polynucleotides of the sequence listing. Column one lists the sequence identification number and column two lists the corresponding tissues and cell types that were found to express the polynucleotide sequences using information from public databases. The number to the right of the tissue or cell type in column two represents the number of entries in the databases listing that tissue or cell type as expressing the sequence of column 1.

In one embodiment, polynucleotides of the invention selectively expressed in tissues may be used as markers to identify these tissues using any technique known to those skilled in the art those skilled in the art such as in situ PCR. Such tissue-specific markers may then be used to identify tissues of unknown origin, for example, forensic samples, differentiated tumor tissue that

has metastasized to foreign bodily sites, or to differentiate different tissue types in a tissue cross-section using immunochemistry. For example, polynucleotides of the invention preferentially expressed in given tissues as indicated in Table VII may be used for this purpose. In addition, the polynucleotide of SEQ ID NO:39 may be used to selectively  
 5 identify liver tissue. The polynucleotide of SEQ ID NO:52 may be used to selectively identify prostate tissue. The polynucleotides of SEQ ID NO:44, 46 and 72 may be used to selectively identify normal or diseased brain tissue.

### EXAMPLE 15B

#### Functional Analysis of Predicted Protein Sequences

10 Following double-sequencing, contiguated sequences were assembled for each of the cDNAs of the present invention and further reanalyzed. The following databases were used in sequence analyses : Genbank (release 117), EMBL (release 62), TrEmbl (release 13.4) Genseq (release 0011) Swissprot (release 38), PIR (release 64). In some cases, more preferred open reading frames differing from the ones previously selected in priority applications are indicated.

15 The polypeptides (SEQ ID NOs :74-123) encoded by the cDNAs were screened for the presence of known structural or functional motifs or for the presence of signatures, small amino acid sequences that are well conserved amongst the members of a protein family. The search was conducted on the Pfam 5.2 database using HMMER-2.1.1 (for info see Sonnhammer et Durbin, <http://www.sanger.ac.uk/Pfam/>), on the BLOCKSPLUS v 11.0 database using emotif (for info see  
 20 Nevill-Manning *et al.*, *PNAS*, 95, 5865-5871, (1998), <http://motif.stanford.edu/EMOTIF>) and on the Prosite 15.0 database using bla (Tatusov, R. L. & Koonin, E. V. CABIOS 10, No. 4) and pfscan (<http://www.isrec.isb-sib.ch/cgi-bin/man.cgi?section=1&topic=pfscan> ).

It should be noted that, in the numbering of amino acids in the protein sequences discussed below, and in Table IX, the first methionine encountered is designated as amino acid number 1, i.e.,  
 25 the leader sequence is not numbered negatively. In the appended sequence listing, the first amino acid of the mature protein resulting from cleavage of the signal peptide is designated as amino acid number 1 and the first amino acid of the signal peptide is designated with the appropriate negative number, in accordance with the regulations governing sequence listings. Each of the references cited in this example are hereby incorporated by reference in their entireties.

30 Table IX lists known biologically structural and functional domains for the cDNA of the present invention corresponding to the sequence identification number indicated in the first column. Column 2 lists the positions of the domains where each domain is represented by x-y where x and y are the start and end positions respectively of a given domain. Column 3 lists the domain designation. Column 4 lists the database from which the domain was identified.

#### 35 Protein of SEQ ID NO: 93 (internal designation 117-007-2-0-C4-FLC)

The protein of SEQ ID NO: 93 encoded by the cDNA of SEQ ID NO:43 found in liver is homologous to a human protein thought to be transmembraneous (Genseq accession number

W88491). In addition, this protein displays homology to alpha-2-HS glycoprotein precursors (fetuins) of human and pigs. The 382-amino-acid-long protein of SEQ ID NO: 93, which is similar in size to fetuins, displays pfam cystatin domains 1 and 2 from positions 37 to 104 and from positions 157 to 254. It also displays the 12 conserved cysteines of this family (positions 36, 93, 5 104, 117, 137, 151, 154, 216, 224, 237, 254 and 368) and a conserved region around the second cysteine (positions 89 to 96). In addition, the potential active site QxVxG is also present in the protein of the invention (positions 198 to 202).

Mammalian fetuins are secreted glycoproteins synthesized in liver and selectively concentrated in bone matrix. Their functions include control of endocytosis, cell proliferation and 10 differentiation, immune response, bone formation and resorption, and apoptosis. More specifically, fetuin levels in human plasma are regulated in the manner of a negative acute phase reactant (Lebreton et al., *J. Clin. Invest.* 64:1118-29 (1979)) and serum levels decline in some cancer patients correlating with impaired cellular immune function (Baskies et al., *Cancer* 45:3050-58 (1980)). During mouse embryogenesis, fetuin mRNA is expressed in a number of developing organs and 15 tissues including the heart, kidney, lung, nervous system and liver (Yang et al., *Biochem. Biophysic. Acta* 1130:149-56 (1992)). Mammalian fetuin present in sub-populations of neurons in the developing central and peripheral nervous system is associated to cell survival (Saunders et al., *Anat. Embryol* 186:477-86 (1992)); Kitchener et al., *Int J. Dev. Neurosci.* 15:717-27 (1997)). Fetuin is able to promote growth in tissue culture (Puck et al. *Proc. Natl. Acad. Sci. U. S. A.*, 59:192-99 20 (1968)), to enhance bone resorption (Coclasure et al., *J. Clin. Endocrinol. Metab.* 66:187-192 (1988)) and to stimulate adipogenesis in cell culture models (Cayatte et al., *J. Biol. Chem.* 265:5883-8 (1990)). Abnormal serum levels of fetuin are associated with alteration in cellular and biochemical properties of bone, Paget's disease, reduced bone quality and osteogenesis imperfecta (for a review see Binkert et al., *J. Biol. Chem.* 274:28514-20 (1999)). Part of the fetuin activities has 25 been shown to depend upon their ability to inhibit the activity of TGF-beta cytokines and bone morphogenetic proteins (BMPs) through direct binding (Demetriou et al., *J. Biol. Chem.* 271:12755-61 (1996); Binkert et al., *J. Biol. Chem.* 274:28514-20 (1999)). These ligands are members of the TGF-beta superfamily comprising proteins belonging to the TGF-beta, activin/inhibin, DPP/VG1, and Mullerian Inhibiting Substance Family families mediating a wide range of biological processes 30 in vertebrates and invertebrates, including regulation of cell proliferation, differentiation, recognition, and death, and thus play a major role in developmental processes, tissue recycling, and repair (J. Wrana and L. Attisano, "Mad-related Proteins in TGF-beta Signaling," *TIG* 12:493-496, 1996; US patent 5,981,483). In addition, fetuins are members of the cystatin superfamily which contains evolutionarily related proteins with diverse functions such as cysteine protease inhibitors, 35 stefins, fetuins and kininogens (see review by Brown and Dziegielewska, *Prot. Science*, 6:5-12 (1997)).

It is believed that the protein of SEQ ID NO: 93 or part thereof is a member of the cystatin superfamily and, as such, plays a role in cellular proteolysis, endocytosis, cell proliferation and differentiation, immune response, bone formation and resorption, and/or apoptosis. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO:93 from 5 positions 37 to 104, 89 to 96, 157 to 254, 198 to 202, and 36 to 368. Other preferred polypeptides of the invention are fragments of SEQ ID NO:93 having any of the biological activity described herein.

An embodiment of the present invention relates to methods of using the protein of the invention or part thereof to identify and/or quantify cytokines of the TGF-beta superfamily, more preferably TGF-1beta, TGF-2beta and BMP-2, BMP-4 and BMP-6 in a biological sample, and thus 10 used in assays and diagnostic kits for the quantification of such cytokines in bodily fluids, in tissue samples, and in mammalian cell cultures. The binding activity of the protein of the invention or part thereof may be assessed using the assay described in Demetriou et al., J. Biol. Chem. 271:12755-61 (1996) or any other method familiar to those skilled in the art. Preferably, a defined quantity of the protein of the invention or part thereof is added to the sample under conditions allowing the 15 formation of a complex between the protein of the invention or part thereof and the cytokine to be identified and/or quantified. Then, the presence of the complex and/or or the free protein of the invention or part thereof is assayed and eventually compared to a control using any of the techniques known by those skilled in the art.

Another embodiment of the invention relates to compositions and methods using the protein 20 of the invention or part thereof to modulate the activity of members of the TGF beta superfamily, preferably members of TGF beta family, members of actin/inhibin family, members of DPP/VG1 family, and members of Mullerian inhibiting substance family, more preferably TGF-1beta, TGF-2beta, BMP-2, BMP-4 and BMP-6, in contexts where the production of such proteins is undesirable.

In a preferred embodiment, the protein of the invention or part thereof is used to inhibit 25 and/or attenuate the effects of cytokines belonging to the TGF beta family, such as TGF-1beta, TGF-2beta and BMP-2, BMP-4 and BMP-6, by blocking the binding of endogenous cytokines to its natural receptor, thereby blocking cell proliferative or inhibitory signals generated by the ligand-receptor binding event. The protein of the invention or part thereof would thereby stimulate immune responses and reduce the deposition of extracellular matrix. Accordingly, the protein of the 30 invention or part thereof, would be particularly suitable for the treatment of conditions such as fibrosis including pulmonary fibrosis, fibrosis associated with chronic liver disease, hepatic veno-occlusive and idiopathic interstitial pneumonitis, kidney disease, and radiotherapy or radiation accidents; proliferative vitreoretinopathy; systemic sclerosis; autoimmune disorders such as rheumatoid arthritis, Graves disease, systemic lupus erythematosus, Wegener's granulomatosis, 35 sarcoidosis, polyarthritis, pemphigus, pemphigoid, erythema multiform, Sjogren's syndrome, inflammatory bowel disease, multiple sclerosis, myasthenia gravis keratitis, scleritis, Type I diabetes, insulin-dependent diabetes mellitus, Lupus Nephritis, and allergic encephalomyelitis;

proliferative disorders including various forms of cancer such as leukemias, lymphomas (Hodgkins and non-Hodgkins), sarcomas, melanomas, adenomas, carcinomas of solid tissue, hypoxic tumors, squamous cell carcinomas of the mouth, throat, larynx, and lung, genitourinary cancers such as cervical and bladder cancer, hematopoietic cancers, head and neck cancers, and nervous system  
5 cancers, benign lesions such as papillomas, atherosclerosis, angiogenesis, and viral infections, in particular HIV infections. The protein of the invention or part thereof may also be used, as an antagonist of cytokines of the TGF-beta family, to elevate blood pressure through the inhibition of hypotension induced by TGF-beta. Methods which lower and/or maintain the level of circulating TGF-beta in a subject may result in a similar pressor effect and may prevent excessive hypotensive  
10 signal generation and resulting hypotension.

In another preferred embodiment, the protein of the invention or part thereof is used to block the normal interaction between activin and its receptor. The protein of the invention or part thereof would thereby stimulate the release of FSH. Accordingly, the protein of the invention or part thereof can be applied to the control of fertility in humans, domesticated animals, and animals of  
15 commercial interest. The action of activin on erythropoiesis can also be modulated by administering a modulating effective amount of the protein of the invention or part thereof. Thus, the protein of the invention or part thereof may be used in the diagnosis and/or treatment of activin-dependent tumors or for enhancing the survival of brain neurons.

In still another preferred embodiment, the protein of the invention or part thereof is used to  
20 modulate bone formation and bone cell differentiation through binding to bone morphogenetic proteins and/or to TGF-beta proteins. Therefore, the protein of the invention or part thereof may be used to repair or heal fractures, treat osteoporosis, address dental problems, and with implants to encourage bone growth. In addition, the protein of the invention or part thereof may be used in disorders where there is too much bone formation (for example, achondroplasia, Paget's disease, and  
25 osteoporosis). The utility of the protein of the invention or part thereof may be further confirmed using binding assays and animal models described in Demetriou et al., *J. Biol. Chem.* 271:12755-61 (1996) and in US Patent 5,981,483.

In still another embodiment, the invention relates to methods and compositions containing the protein of the invention or part thereof to treat and/or prevent the ill-effects of bacterial infection  
30 during pregnancy in mammals, such as spontaneous abortion and maternal death. In a preferred embodiment, the protein of the invention may be used to counteract the effects of the bacterial endotoxin lipopolysaccharide (LPS). The method to use such compositions is described in Dziegielewska and Andersen, *Biol. Neonate*, 74:372-5 (1998).

In another series of embodiments, the protein of the invention, or part thereof may be used  
35 to inhibit proteases, preferably cysteine proteases. Examples of cysteine proteases that may be inhibited by the protein of the invention or part thereof include, but are not limited to, the plant cysteine proteases such as papain, ficin, aleurain, oryzain and actinidin; mammalian cysteine

proteases such as cathepsins B, H, J, L, N, S, T, O, O2 and C, (cathepsin C is also known as dipeptidyl peptidase D), interleukin converting enzyme (ICE), calcium-activated neutral proteases, calpain I and II; bleomycin hydrolase, viral cysteine proteases such as picornian 2A and 3C, aphthovirus endopeptidase, cardiovirus endopeptidase, comovirus endopeptidase, potyvirus  
5 endopeptidases I and II, adenovirus endopeptidase, the two endopeptidases from chestnut blight virus, togavirus cysteine endopeptidase, as well as cysteine proteases of the polio and rhinoviruses; and cysteine proteases known to be essential for parasite lifecycles, such as the proteases from species of Plasmodia, Entamoeba, Onchocera, Trypanosoma, Leishmania, Haemonchus, Dictyostelium, Therileria, and Schistosoma, such as those associated with malaria (*P. falciparum*),  
10 trypanosomes (*T. cruzi*, the enzyme is also known as cruzain or cruzipain), murine *P. vinckei*, and the *C. elegans* cysteine protease. For an extensive listing of cysteine proteases that may be inhibited by the protein or part thereof of the present invention, see Rawlings et al., *Biochem. J.* 290:205-218 (1993). Assays for testing the inhibitory activities of cysteine protease inhibitors are presented in the US patent 5,973,110, using methods for determining inhibition constants well known to those skilled  
15 in the art (see Fersht, *ENZYME STRUCTURE AND MECHANISM*, 2nd ed., W.H. Freeman and Co., New York, (1985)).

Since proteases play an important role in the regulation of many biological processes in virtually all living organisms as well as a major role in diseases, the protein of the invention or part thereof are useful in a wide variety of applications, such as those described in US 6,004,933.

20 An embodiment of the present invention further relates to methods of using the protein of the invention or part thereof to quantify the amount of a given protease in a biological sample, and thus used in assays and diagnostic kits for the quantification of proteases in bodily fluids or other tissue samples, in addition to bacterial, fungal, plant, yeast, viral or mammalian cell cultures. In a preferred embodiment, the sample is assayed using a standard protease substrate. A known  
25 concentration of protease inhibitor is added, and allowed to bind to a particular protease present. The protease assay is then rerun, and the loss of activity is correlated to the protease inhibitor activity using techniques well known to those skilled in the art.

In addition, the protein of the invention or part thereof may be useful to remove, identify or inhibit contaminating proteases in a sample. Compositions comprising the polypeptides of the  
30 present invention may be added to biological samples as a "cocktail" with other protease inhibitors to prevent degradation of protein samples. The advantage of using a cocktail of protease inhibitors is that one is able to inhibit a wide range of proteases without knowing the specificity of any of the proteases. Using a cocktail of protease inhibitors also protects a protein sample from a wide range of future unknown proteases which may contaminate a protein sample from a vast number of  
35 sources. Such protease inhibitor cocktails (see for example the ready to use cocktails sold by Sigma) are widely used in research laboratory assays to inhibit proteases susceptible of degrading a protein of interest for which the assay is to be performed. For example, the protein of the invention or part



thereof is added to samples where proteolytic degradation by contaminating proteases is undesirable. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other protease inhibitors, using techniques well known in the art, to form an affinity chromatography column. A sample containing the undesirable protease  
5 is run through the column to remove the protease. Alternatively, the same methods may be used to identify new proteases.

In a preferred embodiment, the protein of the invention or part thereof may be used to inhibit proteases implicated in a number of diseases where cellular proteolysis occur. In particular, the protein of the invention or part thereof may be useful to inhibit lysosomal cysteine proteases, both in  
10 vivo or in vitro, implicated in a wide spectrum of diseases characterized by tissue degradation including but not limited to arthritis, muscular dystrophy, inflammation, tumor invasion, glomerulonephritis, parasite-borne infections, Alzheimer's disease, periodontal disease, and cancer metastasis.

In another preferred embodiment, the protein of the invention or part thereof may be used to  
15 inhibit exogenous proteases, both in vivo or in vitro, implicated in a number of infectious diseases including but not limited to gingivitis, malaria, leishmaniasis, filariasis, osteoporosis and osteoarthritis, and other bacterial, and parasite-borne or viral infections. In particular, the protein of the invention or part thereof may offer applications in viral diseases where the proteolysis of primary polypeptide precursors is essential to the replication of the virus, as for HIV and HCV.

20 In another preferred embodiment, the protein of the invention or part thereof is used to prevent cells to undergo apoptosis. In a preferred embodiment, the apoptosis active polypeptide is added to an in vitro culture of mammalian cells in an amount effective to reduce apoptosis. For example, inhibiting the activity of apopain, a cysteine protease member of the ICE/CED-3 subfamily involved in apoptosis, attenuates apoptosis in vitro (US patent 5,798,442). Furthermore, the protein  
25 of the invention or part thereof may be useful in the diagnosis, the treatment and/or the prevention of disorders in which apoptosis is deleterious, including but not limited to immune deficiency syndromes (including AIDS), type I diabetes, pathogenic infections, cardiovascular and neurological injury, alopecia, aging, Parkinson's disease and Alzheimer's disease.

Additionally, the protein of the invention or part thereof offer application in the treatment of  
30 inflammation and immune based disorders of the lung, airways, central nervous system and surrounding membranes, eyes, ears, joints, bones, connective tissues, cardiovascular system including the pericardium, gastrointestinal and urogenital systems, the skin and the mucosal membranes. These conditions include infectious diseases where active infection exists at any body site, such as meningitis and salpingitis; complications of infections including septic shock,  
35 disseminated intravascular coagulation, and/or adult respiratory distress syndrome; acute or chronic inflammation due to antigen, antibody and/or complement deposition; inflammatory conditions including arthritis, cholangitis, colitis, encephalitis, endocarditis, glomerulonephritis, hepatitis,

myocarditis, pancreatitis, pericarditis, reperfusion injury and vasculitis. Immune-based diseases include but are not limited to conditions involving T-cells and/or macrophages such as acute and delayed hypersensitivity, graft rejection, and graft-versus-host disease; auto-immune diseases including Type I diabetes mellitus and multiple sclerosis. Bone and cartilage reabsorption as well as  
5 diseases resulting in excessive deposition of extracellular matrix such as interstitial pulmonary fibrosis, cirrhosis, systemic sclerosis, and keloid formation may also be treated with the protein of the invention or part thereof.

Furthermore, the protein of the present invention or part thereof find use in drug potentiation applications. For example, therapeutic agents such as antibiotics or antitumor drugs can be  
10 inactivated through proteolysis by endogenous proteases, thus rendering the administered drug less effective or inactive. Accordingly, the protein of the invention or part thereof may be administered to a patient in conjunction with a therapeutic agent in order to potentiate or increase the activity of the drug. This co-administration may be by simultaneous administration, such as a mixture of the protease inhibitor and the drug, or by separate simultaneous or sequential administration.

15 In addition, protease inhibitors have been shown to inhibit the growth of microorganisms including human pathogenic bacteria. For example, protease inhibitors are able to inhibit growth of all strains of group A streptococci, including antibiotic-resistant strains (Merigan, T. et al (1996) Ann Intern Med 124:1039-1050; Stoka, V. (1995) FEBS. Lett 370:101-104; Vonderfecht, S. et al (1988) J Clin Invest 82:2011-2016; Collins, A. et al (1991) Antimicrob Agents Chemother 35:2444-  
20 2446). Accordingly, the protein of the invention may or part thereof be used as antibacterial agents to retard or inhibit the growth of certain bacteria either in vitro or in vivo. Particularly, the polypeptides of the present invention may be used to inhibit the growth of group A streptococci on non-living matter such as instruments not conducive to other methods of preventing or removing contamination by group A streptococci, and in culture of living plant, fungi, and animal cells.

25 Protein of SEQ ID NO: 86 (internal designation 116-054-3-0-G12-FLC)

The protein of SEQ ID NO: 86 encoded by the cDNA of SEQ ID NO:36 found in liver is homologous to the subunit 2 of NADH dehydrogenase (Genseq accession number Y14556) and to the MLRQ subunit of NADH dehydrogenase (NADH-ubiquinone oxidoreductase, NADH-D or complex I) of bovine, murine and human species (Genbank accession numbers X64897, U59509 and  
30 EMBL accession number U94586 respectively). In addition, the 83-amino-acid-long protein of SEQ ID NO: 86 has a size similar to those of known MLRQ subunits as well as an hydrophobic N-terminal region of 25-30 amino acids.

Complex I is the first of 3 multienzyme complexes located in the mitochondrial membrane that make up the mitochondrial electron transport chain. Complex I accomplishes the first step in  
35 this process by accepting electrons from NADH and passing them through a flavin molecule to ubiquinone which then transfers electrons to the second enzyme complex in the chain.

Complex I contains approximately 40 polypeptide subunits of widely varying size and composition and is highly conserved in a variety of mammalian species including rat, rabbit, cow, and human (Cleeter, M. W. J. and Ragan, C. I. (1985) *Biochem. J.* 230: 739-46). The best characterized complex I is from bovine heart mitochondria and is composed of 41 polypeptides (Walker, J. E. et al. (1992) *J. Mol. Biol.* 226: 1051-72). Seven of these polypeptides are encoded by mitochondrial DNA, while the remaining 34 are nuclear gene products that are imported into the mitochondria. Six of these imported polypeptides are characterized by N-terminal signal peptide sequences which target these polypeptides to the mitochondria and are then cleaved from the mature proteins. A second group of polypeptides lack N-terminal targeting sequences and appear to contain import signals which lie within the mature protein (Walker et al., supra). The functions of many of the individual subunits in NADH-D are largely unknown. The 24-, 51-, and 75-kDa subunits have been identified as being catalytically important in electron transport, with the 51-kDa subunit forming part of the NADH binding site and containing the flavin moiety that is the initial electron acceptor (Ali, S. T. et al. (1993) *Genomics* 18:435-39). The location of other functionally important groups, such as the electron-carrying iron-sulfate centers, remains to be determined. Many of the smaller subunits (<30 kDa) contain hydrophobic sequences that may be folded into membrane-spanning alpha-helices. These subunits presumably are anchored into the inner membrane of the mitochondria and interact via more hydrophilic parts of their sequence with globular proteins in the large extrinsic domain of NADH-D. The remaining proteins are likely to be globular and form part of a domain outside the lipid bilayer. The MLRQ subunit is one of the small (9 kDa) subunits that is nuclear encoded and contains no N-terminal extension to direct the protein into the mitochondrion, thus implying that the import signal should lie into the mature protein (Walker et al. supra). A potential membrane-spanning alpha-helix presumably anchors the MLRQ subunit to the inner membrane of the mitochondria, but the precise function of the subunit is unknown.

Mitochondriocytopathies due to complex I deficiency are frequently encountered and affect tissues with a high-energy demand such as brain (mental retardation, convulsions, movement disorders), heart (cardiomyopathy, conduction disorders), kidney (Fanconi syndrome), skeletal muscle (exercise intolerance, muscle weakness, hypotonia) and/or eye (ophthaloplegia, ptosis, cataract and retinopathy). Complex I is also thought to play a role in the regulation of apoptosis and necrosis. For a review on complex I, see Smeitink *et al.*, *Hum. Mol. Genet.*, 7 : 1573-1579 (1998); Lenaz *et al.*, *Acta Biochem Pol* 46:1-21 (1999); Lee and Wei, *J Biomed Sci* 7:2-15 (2000). In addition, defects and altered expression of complex I are associated with a variety of disease conditions in man, including neurodegenerative diseases, myopathies, and cancer (Singer, T. P. et al. (1995) *Biochim. Biophys. Acta* 1271:211-19; Selvanayagam, P. and Rajaraman, S. (1996) *Lab. Invest.* 74:592-99). Moreover, NADH-D reduction of the quinone moiety in chemotherapeutic agents such as doxorubicin is believed to contribute to the antitumor activity and/or mutagenicity of these drugs (Akman, S. A. et al. (1992) *Biochemistry* 31:3500-6).

It is believed that the protein of SEQ ID NO: 86 is a NADH-ubiquinone oxidoreductase MLRQ-like protein and/or plays a role in mitochondria electron transport. Preferred polypeptides of the invention are fragments of SEQ ID NO: 111 having any of the biological activities described herein

5 An object of the present invention are compositions and methods of targeting heterologous compounds, either polypeptides or polynucleotides to mitochondria by recombinantly or chemically fusing a fragment of the protein of the invention to an heterologous polypeptide or polynucleotide. Preferred fragments are signal peptide, amphiphilic alpha helices and/or any other fragments of the protein of the invention, or part thereof, that may contain targeting signals for mitochondria  
10 including but not limited to matrix targeting signals as defined in Herrman and Neupert, Curr. Opinion Microbiol. 3:210-4 (2000); Bhagwat et al. J. Biol. Chem. 274:24014-22 (1999), Murphy Trends Biotechnol. 15:326-30 (1997); Glaser et al. Plant Mol Biol 38:311-38 (1998); Ciminale et al. Oncogene 18:4505-14 (1999). Such heterologous compounds may be used to modulate mitochondria's activities. For example, they may be used to induce and/or prevent mitochondrial-  
15 induced apoptosis or necrosis. In addition, heterologous polynucleotides may be used for mitochondrial gene therapy to replace a defective mitochondrial gene and/or to inhibit the deleterious expression of a mitochondrial gene.

In another embodiment, the protein of the invention or part thereof is used to prevent cells to undergo apoptosis. In a preferred embodiment, the apoptosis active polypeptide is added to an in vitro  
20 culture of mammalian cells in an amount effective to reduce apoptosis. Furthermore, the protein of the invention or part thereof may be useful in the diagnosis, the treatment and/or the prevention of disorders in which apoptosis is deleterious, including but not limited to immune deficiency syndromes (including AIDS), type I diabetes, pathogenic infections, cardiovascular and neurological injury, alopecia, aging, degenerative diseases such as Alzheimer's Disease, Parkinson's Disease, Huntington's disease, dystonia,  
25 Leber's hereditary optic neuropathy, schizophrenia, and myodegenerative disorders such as "mitochondrial encephalopathy, lactic acidosis, and stroke" (MELAS), and "myoclonic epilepsy ragged red fiber syndrome" (MERRF).

The invention further relates to methods and compositions using the protein of the invention or part thereof to diagnose, prevent and/or treat several disorders in which mitochondrial respiratory  
30 electron transport chain is impaired, or needs to be impaired, including but not limited to mitochondriocytopathies, necrosis, aging, neurodegenerative diseases, myopathies, and cancer. For diagnostic purposes, the expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals. For prevention and/or treatment purposes, the protein of the  
35 invention may be used to enhance electron transport and increase energy delivery using any of the gene therapy methods described herein or known to those skilled in the art.

Moreover, antibodies to the protein of the invention or part thereof may be used for detection of mitochondria organelles and/or mitochondrial membranes using any techniques known to those skilled in the art.

Protein of SEQ ID NO: 111 (internal designation 108-013-5-O-H9-FL)

5       The protein of SEQ ID NO : 111 encoded by the extended cDNA SEQ ID NO: 61 is homologous to the human IHLP lysophospholipase (Genseq accession number W88457) and to a family of lysophospholipases conserved among eukaryotes (yeast, rabbit, rodents and human). In addition, some members of this family (rat :Genbank accession number U97146, rabbit : Genbank accession number U97147) exhibit a calcium-independent phospholipase A2 activity (Portilla *et al*,  
10 *J. Am. Soc. Nephro.*, 9 :1178-1186 (1998)). All members of this family exhibit the active site consensus GX SXG motif of carboxylesterases that is also found in the protein of the invention (position 54 to 58). The protein of the invention also exhibits an emotif alpha/beta hydrolase fold signature from positions 52 to 66. In addition, this protein may be a membrane protein with one transmembrane domain as predicted by the software TopPred II (Claros and von Heijne, *CABIOS*  
15 *applic. Notes*, 10 :685-686 (1994)).

Lysophospholipids are found in very low concentrations in biological membranes. Higher concentrations of lysophospholipids have been shown to disturb membrane conformation, affect the activities of many membrane-bound enzymes and may even lead to cell lysis. In addition, increased lysophospholipid levels were observed in atherosclerosis, inflammation, hyperlipidemia, lethal  
20 dysrhythmias in myocardial ischemia and segmental demyelination of peripheral nerves. Some lysophospholipids, such as lysophosphatidylcholine, may act as lipid second messengers, transducing signals eliciting from membrane receptors. They may also potentiate immune responses and exhibit anti-tumor effects as bactericidal activities (for a review see Wang and Dennis, *Biochim Biophys Acta*; 1439:1-16 (1999)).

25       Lysophospholipase is a widely distributed enzyme which regulates the level of lysophospholipids and occurs in numerous isoforms. These isoforms vary in molecular mass, substrate metabolized, and optimum pH required for activity. Small isoforms, approximately 15-30 kDa, function as hydrolases; large isoforms, those exceeding 60 kDa function both as transacylases and hydrolases. Lysophospholipases are regulated by lipid factors such as acylcarnitine, arachidonic  
30 acid and phosphatidic acid. The expression of IHLP is associated with proliferation and differentiation of cells of the immune system.

The role of lysophospholipases in human tissues has been investigated in various research studies. Selle, H. et al. (1993; *Eur. J. Biochem.* 212:411-16) characterized the role of lysophospholipase in the hydrolysis of lysophosphatidylcholine which causes lysis in erythrocyte  
35 membranes. Similarly, Endresen, M. J. et al. (1993) *Scand. J. Clin. Invest.* 53:733-9 reported that the increased hydrolysis of lysophosphatidylcholine by lysophospholipase in pre-eclamptic women causes release of free fatty acids into the sera. In renal studies, lysophospholipase was shown to

protect  $\text{Na}^+, \text{K}^+$ -ATPase from the cytotoxic and cytolytic effects of cyclosporin A (Anderson, R. et al. (1994) *Toxicol. Appl. Pharmacol.* 125:176-83).

It is believed that the protein of SEQ ID NO:111 or part thereof plays a role in fatty acid metabolism, probably as a phospholipase. Preferred polypeptides of the invention are polypeptides  
5 comprising the amino acids of SEQ ID NO:111 from positions 54 to 58, and 52 to 66. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 111 having any of the biological activities described herein. The hydrolytic activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in Portilla et al., *J Am Soc Nephrol*; 9:1178-1186 (1998) and in the US patent 6,004,792.

10 The invention relates to methods and compositions using the protein of the invention or part thereof to hydrolyze one or several substrates, alone or in combination with other substances. Such substrates are glycerophospholipids, preferably containing an acyl ester bond at the sn-2 position, more preferably lysophosphatidylcholine, lysophosphatidylinositol, lysophosphatidylserine, 1-oleoyl-2-acetyl-sn-glycero-3-phosphocholine, lecithin and lysolecithin. For example, the protein of  
15 the invention or part thereof is added to a sample containing the substrate(s) in conditions allowing hydrolysis, and allowed to catalyze the hydrolysis of the substrate(s). In a preferred embodiment, the hydrolysis is carried out using a standard assay such as those described by Portilla et al., supra and in the US patent 6,004,792.

In a preferred embodiment, the protein of the invention or part thereof may be used to  
20 hydrolyze undesirable phospholipids, both in vitro or in vivo. In particular, the protein of the invention or part thereof may be used as a food additive to improve fat digestibility and to promote growth in animals using methods described in US patent 6,017,530. In another preferred embodiment, the protein of the invention or part thereof may be used to improve the filtration of starch syrup by hydrolyzing the turbidity consisting mainly from phospholipids and resulting from  
25 the production of highly concentrated solutions of glucose isomers using methods described in US patent 5,965,422. In addition, the protein of the invention or part thereof may be used in an enzymatic degumming process to free vegetable oils from phospholipids in order to allow their refining using methods described in US patent 6,001,640. In another preferred embodiment, compositions comprising the protein of the present invention or part thereof are added to samples as  
30 a "cocktail" with other hydrolytic enzymes, such as other phospholipases for example to improve feed utilization in animals (see US patent 6,017,530). The advantage of using a cocktail of hydrolytic enzymes is that one is able to hydrolyze a wide range of substrates without knowing the specificity of any of the enzymes. Using a cocktail of hydrolytic enzymes also protects a sample from a wide range of future unknown contaminants from a vast number of sources. For example, the  
35 protein of the invention or part thereof is added to samples where contaminating substrates is undesirable. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other hydrolytic enzymes, using

techniques well known in the art, to form an affinity chromatography column. A sample containing the undesirable substrate is run through the column to remove the substrate. Immobilizing the protein of the invention or part thereof on a support is particularly advantageous for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the enzyme from the batch of product and subsequent reuse of the enzyme. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by replacing the transmembrane region by a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature. Alternatively, the same methods may be used to identify new substrates.

10 In another embodiment, the protein of the invention or part thereof may be used to identify or quantify the amount of a given substrate in a biological sample. In a preferred embodiment, the protein of the invention or part thereof is used in assays and diagnostic kits for the identification and quantification of substrates in a biological sample.

In still another embodiment, the protein of the invention or part thereof may be used to  
15 diagnose, treat and/or prevent disorders where the presence of substrates is undesirable or deleterious. Such disorders include but are not limited to, cancer, neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, diabetes. In a preferred embodiment, the protein of the invention or part thereof may be administered to a subject to reduce immune response. Although the inventors do not wish to be limited to a particular mechanism of action, it is thought that reduction  
20 would at least protect against lysophospholipid toxicity, deacylate platelet activating factor, and hydrolyze lytic lysophospholipids such as lysophosphatidylcholine which contribute to immune response, and in particular hypersensitivity reactions and immune cell mediated injuries. Such injuries include, but are not limited to, adult respiratory distress syndrome, allergies, asthma, arteriosclerosis, bronchitis, emphysema, hypereosinophilia, myocardial or pericardial inflammation,  
25 rheumatoid arthritis, complications of heart attack, stroke, cancer, hemodialysis, infections, and trauma.

In addition, the protein of the invention or part thereof may be used to identify inhibitors for mechanistic and clinical applications. Such inhibitors may then be used to identify or quantify the protein of the invention in a sample, and to diagnose, treat or prevent any of the disorders where the  
30 protein's activity is undesirable and/or deleterious including but not limited to inflammation, disorders associated with cell proliferation, immune and inflammatory disorders. Disorders associated with cell proliferation include adenocarcinoma, sarcoma, lymphoma, leukemia, melanoma, myeloma, teratocarcinoma, and in particular, cancers of the adrenal gland, bladder, bone, brain, breast, gastrointestinal tract, heart, kidney, liver, lung, ovary, pancreas, paraganglia,  
35 parathyroid, prostate, salivary glands, skin, spleen, testis, thyroid, and uterus. Immune and inflammatory disorders include Addison's disease, AIDS, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative

colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polycystic kidney disease, polymyositis, rheumatoid  
5 arthritis, scleroderma, Sjogren's syndrome, autoimmune thyroiditis.

Moreover, antibodies to the protein of the invention or part thereof may be used for detection of the Golgi apparatus using any techniques known to those skilled in the art.

Protein of SEQ ID NO: 76 (internal designation 105-095-1-0-D10-FLC)

The protein of SEQ ID NO: 76 encoded by the cDNA of SEQ ID NO:26 is homologous to  
10 the human parotid secretory protein HPSP (Genseq accession number W60682 and SEQ ID NO : 124). PSPs are leucine-rich glycoproteins well conserved among the murine, rat, bovine and human species which belongs to the PSP multigenic family with gland specific members which common traits are early and abundant expression. Because it is extremely abundant in saliva, PSP has been proposed as a marker for tissue-specific protein production of salivary glands and appears  
15 coordinately regulated with salivary amylase. PSP is also expressed although to a lesser extent in murine lacrimal glands. Although its function remains unknown, it was shown to bind to bacteria in exocrine secretions and was proposed to have antibacterial activity (Robinson *et al.*, *Am J Physiol* 272:G863-G871 (1997)). Antagonists of this protein may be used to treat cancer and autoimmune diseases particularly of secretory or gastrointestinal tissue.

20 It is believed that the protein of SEQ ID NO: 76 or part thereof plays a role in the defense against pathogens, preferably pathogens present in the oral and gastrointestinal tracts. Preferred polypeptides of the invention are fragments of SEQ ID NO: 76 having any of the biological activity described herein. The activity of the protein of the invention or part thereof on pathogens may be assessed using techniques well known to those skilled in the art including those described in  
25 Robinson et al, supra.

In one embodiment, the present invention relates to methods and compositions using the protein of the invention or part thereof to detect bacteria in biological fluids, foods, water, air, solutions and the like. For example, the protein of the invention or part thereof is added to a sample containing bacteria and allowed to bind to such bacteria using any method known to those skilled in  
30 the art including those described in Robinson et al, supra. Then, the protein may be detected using any method known to those skilled including using an antibody able to bind to the protein of the invention or part thereof, or using another polypeptide fused to the protein of the invention or part thereof that may be detected directly, such as the green fluorescent protein, or through binding to a specific antibody. In a preferred embodiment, the protein of the invention or part thereof is used in  
35 assays and diagnostic kits for the detection of exogenous pathogens in bodily fluids, tissue samples or cell cultures. In another preferred embodiment, the protein of the invention or part thereof may be used to decontaminate samples. For example, the protein of the invention or part thereof may be



bound to a chromatographic support using techniques well known in the art, to form an affinity chromatography column. A sample containing the undesirable contaminant is run through the column in order to be removed. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly for those embodiments in which the method is to be practiced  
5 on a commercial scale. This immobilization facilitates the removal of the protein of the invention from the batch of product and its subsequent reuse. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature.

10 In another embodiment, the invention related to methods and compositions using the protein of the invention or part thereof to retard and/or inhibit the growth of pathogens, preferably bacteria, more preferably *Listeria* and *Streptococci*, and *Actinobacilli*, either in vitro or in vivo using any methods and techniques known to those skilled in the art, alone or in combination with other antimicrobial substances. For example, the protein of the invention or part thereof may be used to  
15 disinfect aqueous samples or materials, or as a food preservative. In a preferred embodiment, compositions comprising the protein of the present invention or part thereof are added to samples or materials as a "cocktail" with other antimicrobial substances to decontaminate samples. The advantage of using such a cocktail is that one is able to decontaminate samples without knowing the specificity of any of the antimicrobial substances. Using such a cocktail also protects a sample or  
20 material from a wide range of future unknown contaminants from a vast number of sources.

In another embodiment, the invention relates to methods and compositions using the protein of the invention or part thereof as a marker protein to selectively identify tissues, preferably salivary glands and lacrimal glands. For example, the protein of the invention or part may be used to synthesize specific antibodies using any techniques known to those skilled in the art including those  
25 described therein. Such tissue-specific antibodies may then be used to identify tissues of unknown origin, for example, forensic samples, differentiated tumor tissue that has metastasized to foreign bodily sites, or to differentiate different tissue types in a tissue cross-section using immunochemistry.

Protein of SEQ ID NO: 120 (internal designation 108-019-5-0-F5-FLC)

30 The protein of SEQ ID NO: 120 encoded by the cDNA of SEQ ID NO: 70 is homologous to human proteins either thought to be a transmembrane proteolipid protein down regulated upon cell differentiation induced by sodium butyrate (Genbank accession number AF057306) or described as the alternatively spliced chemokine-like factor 2 (Genbank accession number AF135380).

Proteolipids are a class of hydrophobic membrane proteins characterized in part by their  
35 capacity to assume conformations compatible with solubility in organic solvents and in water (Sapirstein V. S. et al (1983) *Biochemistry* 22:3330-3335). This amphipathic character of proteolipids explains their participation in transmembrane ion movement. Proteolipids are

components of ion channel and transport systems, such as H<sup>+</sup> channels (Arai H. et al (1987) J Biol Chem 262:11006-11011), Ca<sup>2+</sup> channels (Eytan G. D. et al (1977) J Biol Chem 252: 3208-3213) and the C (membrane channel) subunit of the vacuolar H<sup>+</sup>-ATPase (Nelson H. et al (1990) J Biol Chem 265: 20390-20393).

5       The latter proteolipid, also known as ductin, is also associated with gap junctions. Gap junctions are the relatively large pores which allow free diffusion of ions across biological membranes (Finbow M. E. et al (1995) Bioessays 17:247-255). Altered gap-junction intercellular communication (GJIC) may play an essential role in cancer development. A lack of GJIC has been observed between transformed and neighboring normal cells (Trosko et al (1990) Radiation Res  
10 123:241-251). A decrease in GJIC has also been observed within tumor cells (Krutovskikh et al (1991) Carcinogenesis 12:1701-1706).

Proteolipids are also involved in membrane vesicular trafficking. Due to their lipid-like properties, proteolipids destabilize lipid bilayers and promote membrane vesicle fusion. Such proteolipid-assisted events may include the fusions and fissions of the nuclear membrane,  
15 endoplasmic reticulum, Golgi apparatus, and various inclusion bodies (peroxisomes, lysosomes, etc).

Human T-lymphocyte maturation-associated protein (MAL), a 153 amino acid proteolipid, has been localized to the endoplasmic reticulum (ER) of T-lymphocytes, where it mediates the fusion of ER-derived vesicles and Golgi cisterna (Rancano C. et al (1994) J Biol Chem 269:8159-  
20 8164). A canine MAL homologue, VIP17, is involved in the sorting and targeting of proteins between the Golgi complex and the apical plasma membrane (Zacchetti D. et al (1995) FEBS Lett 377:465-469). A rat MAL homologue, rMAL, is expressed in the myelinating cells of the nervous system including oligodendrocytes and Schwann cells. The rMAL protein serves as a gap junction component and plays a role in myelin compaction (Schaaeren-Wiemers N. et al (1995) J. Neurosci  
25 5753-5764).

Plasmolipin from rat is a proteolipid localized to plasma membranes in kidney and brain. It has 157 amino acids and, based on hydropathy plots and secondary structure predictions, consists of four alpha-helical transmembrane domains (I through IV) of 20-22 amino acids in length. Transmembrane domains III and IV contain hydroxyl groups which may contribute to an aqueous  
30 channel. Domains I through III are connected by short hydrophilic segments of 9-11 amino acids in length, and domains III and IV are connected by a longer hydrophilic segment of 20 amino acids. The small size and high hydrophobicity of plasmolipin constrains the distribution of its transmembrane regions such that the four transmembrane alpha-helices form an antiparallel bundle, and both the amino- and carboxy-termini face the cytoplasm. This structural model defines the  
35 growing class of small hydrophobic transport-related proteolipids containing four-helix transmembrane segments, such as the MAL homologues (Rancano et al, supra), and the vacuolar H<sup>+</sup>-ATPase C subunit (Nelson et al, supra).

In rat brain, plasmolipin is localized to myelinated nerve tracts, and its expression increases markedly with the onset of myelination (Fischer I. et al (1991) *Neurochem Res* 28:81-89). The distribution of plasmolipin within myelin appears to include regions active in membrane recycling. Endocytotic coated vesicles isolated from myelinated tracts are enriched with plasmolipin  
5 (Sapirstein V. S. (1994) *J Neurosci Res* 37:348-358). Incorporation of the purified rat plasmolipin protein into lipid bilayers induces voltage-dependent K<sup>+</sup> channel formation, suggesting it may function in vivo as a pore or channel (Tosteson M. T. et al (1981) *J Membr Biol* 63:77-84). Channel formation involved the trimerization of the plasmolipin molecule. The oligomerization model of the plasmolipin molecule portrays transmembrane domains III and IV as walls of the channel, consistent  
10 with the presence of hydroxyl groups in these domains (Sapirstein et al (1983) *supra*). The putative role of rat plasmolipin in transport suggests its function may be in the fluid volume regulation of the myelin complex (Fischer et al (1994), *supra*).

Proteolipids are involved in membrane trafficking, gap junction formation, ion transport and cellular fluid volume regulation. The selective modulation of their expression may provide a means  
15 for the regulation of vesicle trafficking or the formation of channels or gap junctions in normal as well as acute and chronic disease situations.

It is believed that the protein of SEQ ID NO: 120 or part thereof plays a role membrane trafficking, gap junction formation, ion transport and/or cellular fluid volume regulation. Preferred polypeptides of the invention are fragments of SEQ ID NO: 120 having any of the biological activity described  
20 herein. The ability of the protein of the invention or part thereof to form pore and/or to destabilize lipid bilayers may be assessed using techniques well known to those skilled in the art including those described in US patent 5,843,714.

The invention relates to methods and compositions using the protein of the invention or part thereof to promote membrane vesicle fusion both in vitro and in vivo.

25 In an embodiment, the protein of the invention or part thereof is used to facilitate exocytosis. For example, the protein of the invention or part thereof may be used to increase the release of chemokines involved in cell migration, proteases which are active in inflammation or other similar activities involving endothelial cells, fibroblasts, lymphocytes, etc. Accordingly, the protein of the invention or part thereof may be used to diagnose, treat and/or prevent disorders associated with  
30 abnormal membrane trafficking including but not limited to viral or other infections, traumatic tissue damage, hereditary diseases such as arthritis or asthma, invasive leukemias and lymphomas.

In another embodiment, the protein of the invention or part thereof may be used to promote vesicle fusion for drug delivery. The protein of the invention or part thereof may be incorporated into liposomes or artificial vesicles with a drug of interest and then used to promote vesicle fusion  
35 for drug delivery.

In another embodiment, antibodies to the protein of the invention or part thereof may be used for detection of membranes and/or gap junctions using any techniques known to those skilled

in the art. In a preferred embodiment, the protein of the invention or part thereof may be used to diagnose disorders associated with altered intercellular communication, more preferably altered gap-junction communication, including but not limited to cardiac arrhythmia.

Protein of SEQ ID NO: 74 (internal designation 105-016-3-0-E3-FLC)

5       The 325-amino-acid-long protein of SEQ ID NO : 74 encoded by the cDNA of SEQ ID NO: 24 shows homology over the whole length of the 332-amino-acid-long murine neural proliferation differentiation and control 1 protein or NPDC-1 (Genbank accession number X67209) which is thought to play an important role in the control of neural cell proliferation and differentiation as well as in cell survival by interacting with cell cycle regulators such as E2F-1 (Galiana *et al.*, *Proc. Natl. Acad. Sci. USA* 92:1560-1564 (1995); Dupont *et al.*, *J. Neurosci. Res.* 51:257-267 (1998)).

10       It is believed that the protein of SEQ ID NO: 74 or part thereof plays a role in cell proliferation and differentiation. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 74 from positions 1 to 81, and 129 to 308. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 74 having any of the biological activity  
15 described herein. The activity of the protein of the invention or part thereof on cellular proliferation and differentiation may be assessed using techniques well known to those skilled in the art including those described in Galiana et al, supra.

20       In one embodiment, the invention related to methods and compositions using the protein of the invention or part thereof to inhibit cellular proliferation, preferably neuronal cell proliferation, using any methods and techniques known to those skilled in the art including those described in Galiana et al, supra.

25       In another embodiment, the protein of the invention or part thereof, may be used to diagnose, treat and/or prevent several disorders linked to cell proliferation and differentiation including, but not limited to cancer and neurodegenerative disorders such as Parkinson's or Alzheimer's diseases. For diagnostic purposes, the expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals.

Protein of SEQ ID NO: 75 (internal designation 105-031-3-0-D6-FLC)

30       The protein of SEQ ID NO: 75 encoded by the cDNA of SEQ ID . NO:25 exhibits homology to a murine putative sialyltransferase protein (TREMBL accession number O88725). Although sialyltransferases have virtually no sequence homology, they display the features of type II transmembrane proteins with a short N -terminal cytoplasmic tail, a 16-20 amino acid signal-anchor domain, and an extended stem region which is followed by the large C-terminal catalytic domain (Weinstein, J. et al., *J. Biol. Chem.* 262, 17735-17743, 1987; Paulson, J. C. et al., *J. Biol. Chem.*  
35 264,17615-17618, 1989).

      The protein of SEQ ID NO: 75 displays the two conserved motifs of the sialyltransferase protein family, namely the centrally located sialylmotifL (positions 73 to 120) thought to be

involved in the recognition of the sugar nucleotide donor common to all sialyltransferases and the sialylmotifs (positions 211 to 233) thought to be the catalytic site and located in the C-terminus of the protein. Furthermore, the 302-amino-acid long protein of SEQ ID NO: 75 has a size similar to the one of the members of the sialyltransferase family. In addition, the protein of the invention has a  
5 predicted transmembrane structure. Indeed, it contains 2 potential transmembrane segments (positions 7 to 27 and 206 to 226, underlined in figure 12) as predicted by the software TopPred II (Claros and von Heijne, *CABIOS applic. Notes*, 10 :685-686 (1994)).

Sialyltransferases are glycosyl transferases found primarily in the Golgi apparatus and also in body fluids such as breast milk, colostrum and blood. They are responsible for the terminal  
10 sialylation of carbohydrate groups of glycoproteins, glycolipids and oligosaccharides widely distributed in animal tissues. Sialic acids play important roles in the biological functions of carbohydrate structures because of their terminal position. Sialyltransferases are indeed involved in a large variety of biological processes such as cell-cell communication, cell-matrix interactions, maintenance of serum glycoproteins in the circulation, and so on (Sjoberg et al., *J. Biol. Chem.*  
15 271:7450-7459 (1996); Tsuji, *J. Biochem.* 120:1-13 (1996)). A variety of biological phenomena are associated with recognition of sialosides, including viral replication, escape of immune detection, and cell adhesion (Schauer, R. *Trends Biochem. Sci.* 1985, 10, 357-360; *Biology of the Sialic Acids* ed. A. Rosenberg, Plenum Press, New York, 1995). For example, suppressed antibody production was observed in alpha-2, 6-sialyltransferase knockout mice (Muramatsu, *J. Biochem.* 127:171-6  
20 (2000). In addition, carbohydrate structures have been shown to influence proteins' stability, rate of in vivo clearance from blood stream, rate of proteolysis, thermal stability and solubility. Changes in the oligosaccharide portion of cell surface carbohydrates have been noted in cells which have become cancerous.

It is believed that the protein of SEQ ID NO: 75 or part thereof plays a role in the  
25 biosynthesis of sialyl-glycoconjugates, probably as a sialyltransferase. Thus, the protein of the invention or part thereof is thought to be involved in cell-cell communication, cell-matrix interactions, maintenance of serum glycoproteins in the circulation, viral replication, escape of immune detection, and cell adhesion. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO:75 from positions 73 to 120, and from position 211 to  
30 233. Other preferred polypeptides of the invention are fragments of SEQ ID NO:75 having any of the biological activity described herein. The sialyltransferase activity of the protein of the invention or part thereof may be assayed using any other technique known to those skilled in the art including those described in Sadler et al., *J. Biol. Chem.*, 254:4434-4443 (1979) or US patents 5,827,714 and 6,017,743.

35 One object of the present invention are compositions and methods of targeting heterologous polypeptides to the Golgi apparatus by recombinantly or chemically fusing a fragment of the protein of the invention to an heterologous polypeptide. Preferred fragments are signal peptide,

transmembrane domains, the proline-rich region comprised between positions 31 and 67, tyrosine containing regions and/or any other fragments of the protein of the invention, or part thereof, that may contain targeting signals for the Golgi apparatus including but not limited to proline-rich regions (Ugur and Jones, *Mol Cell Biol* 11:1432-32 (2000), Picetti and Borrelli, *Exp Cell Res* 5 255:258-69 (2000)), tyrosine-based Golgi targeting signal region (Zhan et al., *Cancer Immunol Immunother* 46:55-60 (1998); Watson and Pessin *J. Biol. Chem.* 275:1261-8 (2000); Ward and Moss, *J. Virol.* 74:3771-80 (2000) or any other region as defined in Munro, *Trends Cell Biol.* 8:11-15 (1998); Luetterforst et al., *J. Cell. Biol.* 145:1443-59 (1999); Essl et al., *FEBS Lett.* 453:169-73 (1999).

10 Sialylated compounds have considerable potential both as therapeutics and as reagents for clinical assays. However, synthesis of glycosylated compounds of potential commercial and/or therapeutic interest is difficult because of the very nature of the saccharide subunits. A multitude of positional isomers in which different substituent groups on the sugars become involved in bond formation, along with the potential formation of different anomeric forms, are possible. As a result  
15 of these problems, large scale chemical synthesis of most carbohydrates is not possible due to economic considerations arising from the poor yields of desired products. Enzymatic synthesis using glycosyl transferases such as sialyltransferases provides an alternative to chemical synthesis of carbohydrates. Enzymatic synthesis using glycosidases, glycosyl transferases, or combinations thereof, have been considered as a possible approach to the synthesis of carbohydrates. As a matter  
20 of fact, enzyme-mediated catalytic synthesis would offer dramatic advantages over the classical synthetic organic pathways, producing very high yields of carbohydrates economically, under mild conditions in aqueous solutions, and without generating notable amounts of undesired side products. To date, such enzymes are however difficult to isolate, especially from eukaryotic, e.g., mammalian sources, because these proteins are only found in low concentrations, and tend to be membrane-  
25 bound. In addition to being difficult to isolate, the acceptor (peptide) specificity of glycosyl transferases is poorly understood. Thus, there is a need for obtaining recombinant glycosyl transferase, including sialyltransferases, that could be produced in very large amounts.

Thus, the invention related to methods and compositions using the protein of the invention or part thereof to synthesize glycosylated compounds, either glycoproteins, glycolipids, or  
30 oligosaccharides, more particularly sialylated compounds. If necessary, the protein of the invention or part thereof may be produced in a soluble form by removing its transmembrane domains and/or its Golgi retention signal using any of the methods skilled in the art including those described in US patent 5,776,772. For example, the protein of the invention or part thereof is added to a sample containing sialic acid and a substrate compound in conditions allowing glycosylation, more  
35 particularly sialylation and allowed to catalyze the glycosylation of this compound. In a preferred embodiment, the enzymatic reaction carried out by the protein of the invention is part of a series of other chemical and/or enzymatic reactions aiming at the synthesis of complex glycosylated

compounds, such as the ones described in US patents 5,409,817 and 5,374,541. In another preferred embodiment where the method is to be practiced on a commercial scale, it may be advantageous to immobilize the glycosyl transferase on a support. This immobilization facilitates the removal of the enzyme from the batch of product and subsequent reuse of the enzyme. Immobilization of glycosyl transferases can be accomplished, for example, by removing from the transferase its membrane-binding domain, and attaching in its place a cellulose-binding domain. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature.

In another embodiment, the present invention relates to processes and compositions for producing glycosylated compounds, preferably sialylated compounds, wherein a cell is genetically engineered to produce the protein of the invention or part thereof and used in combination with one or several other cells able to produce the donor substrate for the protein of the invention. Preferably, a bacteria is engineered to express the protein of the invention and used with recombinant bacteria expressing enzymes able to synthesize cytidine 5'-monophospho-N-acetyl neuraminic acid (CMP-NeuAc). The methods for performing the above bacterial coupling process and making the above compositions are carried using the methods known in the art and described in Endo et al., Appl. Microbiol. Biotechnol. 53:257-61, (2000).

Another embodiment of the present invention relates to a process and compositions for controlling the glycosylation of proteins in a cell wherein an insect, plant, or animal cell is genetically engineered to produce one or more enzymes which provide internal control of the cell's glycosylation mechanism. Preferably, the invention relates to a Chinese hamster ovary (CHO) cell line that is genetically engineered to produce a sialyltransferase of the present invention either alone or in combination with other sialyltransferases. This supplemental sialyltransferase modifies the CHO glycosylation machinery to produce glycoproteins having carbohydrate structures which more closely resemble naturally occurring human glycoproteins. The methods for performing the above process and making the above compositions are carried using the methods known in the art and described in U.S. Patent No. 5,047,335.

The invention further relates to glycosylated compounds, preferably sialylated compounds, obtained using any of the processes described herein using the protein of the invention or part thereof. Such compounds may be used in the diagnosing, prevention and/or treating of disorders in which the recognition of such compounds is impaired or needs to be impaired. These disorders include, but are not limited to, cancer, cystic fibrosis, ulcer, inflammation and immune based disorders, including autoimmune disorders such as arthritis, fertility disorders, and hypothyroidism. These conditions include infectious diseases where active infection exists at any body site, such as meningitis and salpingitis; complications of infections including septic shock, disseminated intravascular coagulation, and/or adult respiratory distress syndrome; acute or chronic inflammation due to antigen, antibody and/or complement deposition; inflammatory conditions including arthritis,

chalangitis, colitis, encephalitis, endocarditis, glomerulonephritis, hepatitis, myocarditis, pancreatitis, pericarditis, reperfusion injury and vasculitis. Immune-based diseases include but are not limited to conditions involving T-cells and/or macrophages such as acute and delayed hypersensitivity, graft rejection, and graft-versus-host disease; auto-immune diseases including Type I diabetes mellitus and multiple sclerosis. In a preferred embodiment, these glycosylated compounds or derivatives thereof may be used as pharmacological agents to trap pathogens or endogenous ligands thus reducing the binding of pathogens or endogenous ligands to the endogenous glycosylated compounds. For example, such compounds may be used to prevent and/or inhibit the adhesion of cancer cells to inner wall of blood vessel or aggregation between cancer cells and platelets, thus reducing cancer metastasis, to prevent and/or inhibit the adhesion of neutrophils to blood vessels endothelial cells, thus reducing inflammation. Other disorders include infections in which recognition of a glycosylated product is essential to the development of the infection. Such infections include, but are not limited to, those caused by *Vibrio cholerae*, *Escherichia Coli*, *Salmonella*, and the influenza virus. In a preferred embodiment, such compounds, preferably sialyl lactose, are used as neutralizers for enterotoxins from bacteria such as *Vibrio cholerae*, *Escherichia Coli*, and *Salmonella* as described in U.S. Pat. No. 5,330,975. In another preferred embodiment, such compounds, preferably galactose oligosaccharides, are used to diagnose, identify and inhibit the adherence of uropathogenic bacteria to red blood cells (U.S. Pat. No. 4,657,849). In another preferred embodiment, such compound, preferably oligosaccharides, are used as gram positive antibiotics and disinfectants (U.S. Pat. Nos. 4,851,338 and 4,665,060). In another embodiment, such compounds, preferably sialyl lactose, may be used for the treatment of arthritis and related autoimmune diseases (see, U.S. Pat. No. 5,164,374). In another embodiment, such compounds, preferably sialylalpha(2,3)galactosides, sialyl lactose and sialyl lactosamine, may be used for the treatment of ulcers. Phase I clinical trials have begun for the use of the former compound in this capacity. (Balkonen, et al., *FEMS Immunology and Medical Microbiology* 7:29 (1993) and *BioWorld Today*, p. 5, Apr. 4, 1995). In addition, such compounds, preferably sialyl lactose, may be used as food supplement, for instance in baby formula.

In addition, the protein of the invention or part thereof may be used in the development of inhibitors of glycosyl transferase, more particularly inhibitors of sialyltransferases and sialidases, for mechanistic and clinical applications (Taylor, G. *Curr. Opin. Struc. Biol.* 1996, 6, 830-837; Colman, P. M., *Pure Appl. Chem.* 1995, 67, 1683-1688; Bamford, M. J. *J. Enz. Inhib.* 1995, 10, 1-16; Khan, S. H. & Matta, K. L. In *Glycoconjugates, Composition, Structure, and Function*. pp361-378. ed., Allen, H. J. & Kisailus, E. C. Marcel Dekker, Inc. New York, 1992, Thorne-Tjomsland et al., *Transplantation* 69:806-8, (2000); Basset et al, *Scand. J. Immunol.* 51:307-11 (2000)).

The invention further relates to methods and compositions using the protein of the invention or part thereof to diagnose, prevent and/or treat several disorders in which recognition of glycosylated compounds, preferably of sialylated compounds, is impaired or needs to be impaired.



For diagnostic purposes, the expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals. For prevention and/or treatment purposes, inhibiting the endogenous expression of the protein of the invention using any of the antisense or triple helix methods described herein may be used to reduce the production of glycosylated compounds detrimental to the organism in any of the disorders described above.

Protein of SEQ ID NOs: 104 (internal designation 108-008-5-O-C5-FL)

The protein of SEQ ID NO: 104 encoded by the cDNA of SEQ ID NO: 54 exhibits homology over the whole length to the murine recombination activating gene 1 inducing protein found in stromal cell (Genbank accession number X96618). The amino acid residues are identical except for the positions 6, 7, 10-13, 17, 25, 34-35, 42, 51, 56, 62, 68, 71, 74, 78, 91, 93, 95-96, 106, 121-122, 151-152, 159, 162-163, 170-171, 176-177, 188, 190, 192, 196, 199, 202-203, 206, 210, 215 and 217 of the 221 amino acid long matched protein. This protein with 4 potential transmembrane segments facilitates gene activation of RAG-1 which is involved in the recombination of V(D)J segments in T cells (Tagoh et al., *Biochem Biophys Res Comm* 221:744-749 (1996); Muraguchi et al, *Leuk Lymphoma*, 30 :73-85 (1998)).

It is believed that the protein of SEQ ID NO: 104 may play a role in lymphocyte repertoire formation. Preferred polypeptides of the invention are fragments of SEQ ID NO: 74 having any of the biological activity described herein. The activity of the protein of the invention or part thereof on the induction of RAG expression may be assessed using techniques well known to those skilled in the art including those described in Tagoh et al, supra.

In an embodiment, antibodies to the protein of the invention or part thereof may be used as markers for haematopoietic precursors, preferably precursors for B and T cells.

In another embodiment, the protein of the invention or part thereof, may be used to diagnose, treat and/or prevent immunological disorders including, but not limited to Ommen's syndrome, acute and delayed hypersensitivity, graft rejection, and graft-versus-host disease; auto-immune diseases including Type I diabetes mellitus and multiple sclerosis, lymphoid neoplasia including non Hodgkins' lymphoma, ALL and CLL. For diagnostic purposes, the expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals. In another embodiment, the protein of the invention or part thereof may also be used to modulate the immune response to pathogens.

Protein of SEQ ID NO: 87 (internal designation 116-073-4-0-C8-FLC)

The protein of SEQ ID NO : 87 encoded by the cDNA of SEQ ID NO:37 shows homology over the whole length of the widely conserved family of lysozyme C precursors (fish, bird, and mammals). In particular, the protein of the invention displays 17 out of the 20 amino acids conserved among all known lysozyme C proteins at positions 115, 117, 123, 137, 141, 144, 146,

150, 151, 162, 166, 180, 181, 194, 197, 201 and 213 (Prager and Jollès, *Lysozymes: model enzymes in biochemistry and biology*, ed. Jollès, 9-321 (1996)). In addition, this protein displays the characteristic signature of the family 22 of glycosyl hydrolases (PROSITE signature from positions 162 to 185, eMotif signatures from positions 183 to 202 and from positions 111 to 120), which  
5 contain the evolutionary related alpha-lactalbumin, the regulatory subunit of lactose synthetase, and the bacteriolytic defensive enzymes lysozyme C (Qasba and Kumar, *Crit. Rev. Biochem. Mol. Biol.* 32:255-306 (1997)). Furthermore, the cDNA of SEQ ID NO:37 seems to be preferentially expressed in testis (Table VII) and in germ cells tumors (Table VIII).

Lysozyme, an ubiquitous protein secreted in most body secretions, is defined as 1,4-beta-N-  
10 acetylmuramidases which cleave the glycoside bond between the C-1 of N-acetyl-muramic acid and the C-4 of N-acetylglucosamine in the peptidoglycan of bacteria. It has various therapeutic properties, such as antiviral, antibacterial, anti-inflammatory and antihistaminic effects. The activity of the lysozyme as an anti-bacterial agent appears to be based on both its direct bacteriolytic activity and also on stimulatory effects in connection with phagocytosis of polymorphonuclear leucocytes and macrophages  
15 (Biggar and Sturgess, *J. M. Infect Immunol.* 16: 974-982 (1977); Thacore and Willet, *Am. Rev. Resp. Dis.* 93: 786-790 (1966); Klockars and Roberts, *P. Acta Haematol* 55: 289-292 (1976)). Lysozyme has proven to be not only a selective factor but also an effective factor against microorganisms of the mouth (Iacono et al, *J. J. Infect. Immunol.* 29: 623-632 (1980)). Lysozyme can also kill pathogens by acting synergistically with other proteins such as complement or antibody to lyse pathogenic cells. Lysozyme,  
20 also inhibits chemotaxis of polymorphonuclear leukocytes and limits the production of oxygen free radicals following an infection. This limits the degree of inflammation, while at the same time enhances phagocytosis by these cells. Other postulated functions of lysozyme include immune stimulation (Jolles, *P. Biomedicine* 25: 275-276 (1976) Ossermann, E. F. *Adv. Pathobiol* 4: 98-102 (1976)) and immunological and non-immunological monitoring of host membranes for any neoplastic  
25 transformation (Jolles, *P. Biomedicine* 25: 275-276 (1976); Ossermann, E. F. *Adv. Pathobiol* 4: 98-102 (1976)). Lysozyme may thus be used in a wide spectrum of applications (see US patent 5,618,712). Determination of the lysozymes from serum and/or urine is used to diagnose various diseases or as an indicator for their development. In acute lymphoblastic leukaemia the lysozyme serum level is significantly reduced, whereas in chronic myelotic leukaemia and in acute monoblastic and  
30 myelomonocytic leukaemia the lysozyme concentration in the serum is greatly increased. The therapeutically effective use of lysozyme is possible in the treatment of various bacterial and virus infections (Zona, Herpes zoster), in colitis, various types of pain, in allergies, inflammation and in pediatrics (the conversion of cows milk into a form suitable for infants by the addition of lysozyme).

It is believed that the protein of SEQ ID NO: 87 or part thereof plays a role in glycoprotein  
35 and/or peptidoglycan metabolism, probably as a glycosyl hydrolase of family 22. Thus, the protein of the invention or part thereof may be involved in immune and inflammatory responses and may have antiviral, antibacterial, anti-inflammatory and/or anti-histaminic functions. Preferred polypeptides of the

invention are polypeptides comprising the amino acids of SEQ ID NO:87 from positions 70 to 215, 111 to 120, 183 to 202, and 162 to 185. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 87 having any of the biological activities described herein. The glycolytic activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in Gold and Schweiger, M. *Methods in Enzymology*, Vol. XX, Part C pp. 537-542, Ed. Moldave, Academic Press, New York and London, 1971 and in the US patent 4,255,517.

The invention relates to methods and compositions using the protein of the invention or part thereof to hydrolyze one or several substrates, alone or in combination with other substances, preferably antiviral, antifungal and/or antibacterial substances including but not limited to immunoglobulins, lactoferrin, betalysin, fibronectin, and complement components. Such substrates are glycosylated compounds, preferably containing beta-1-4-glycoside bonds, more preferably containing beta-1-4-glycoside bonds between n-acetylmuraminic acid and n-acetylglucosamine. For example, the protein of the invention or part thereof is added to a sample containing the substrate(s) in conditions allowing hydrolysis, and allowed to catalyze the hydrolysis of the substrate(s). In a preferred embodiment, the hydrolysis is carried out using a standard assay such as those described by Gold and Schweiger, *supra*, and US patents 5,871,477 and 4,255,517. In a preferred embodiment, the protein of the invention or part thereof may be used to lyse recombinant bacteria in order to recover the recombinant DNA, the recombinant protein of interest, or both using, for example, any of the assays described in Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989).

In an embodiment, the protein of the invention or part thereof is used to hydrolyze contaminating substrates in an aqueous sample or onto a material, preferably glassware and plasticware. In particular, the protein of the invention or part thereof may be used as a disinfectant in dental rinse, in protection of aqueous systems or in preparing material for medical applications using any of the methods and compositions described in US patents 5,069,717, 4,355,022 and 5,001,062. In a preferred embodiment, the protein of the invention is used as a host resistance factor in infants' formulas to convert cow's milk into a form more suitable for infants as described in US patent 6,020,015. In another preferred embodiment, the protein of the invention or part thereof may be used as a food preservative (see Hayashi et al., *Agric. Biol. Chem. (European Edition of Japanese Journal of Agriculture, Biochemistry and Chemistry)*, Vol. 53, pp. 3173-3177, 1989). In addition, the protein of the invention or part thereof may be used to clarify xanthan gum fermented broth for applications in food and in cosmetic industries using the method described in US patent 5,994,107. In another preferred embodiment, compositions comprising the protein of the present invention or part thereof are added to samples or materials as a "cocktail" with other antimicrobial substances, preferably antibiotics or hydrolytic enzymes such as those described in US patents 5,458,876 and 5,041,326 to decontaminate the samples. For example, the protein of the invention or part thereof may be used in place or in combination with antibiotics in cell cultures. The advantage of using a

cocktail of hydrolytic enzymes is that one is able to hydrolyze a wide range of substrates without knowing the specificity of any of the enzymes. Using a cocktail of hydrolytic enzymes also protects a sample or material from a wide range of future unknown contaminants from a vast number of sources. For example, the protein of the invention or part thereof is added to samples where  
5 contaminating substrates is undesirable. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other hydrolytic enzymes, using techniques well known in the art, to form an affinity chromatography column. A sample containing the undesirable substrate is run through the column to remove the substrate. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly  
10 for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the enzyme from the batch of product and subsequent reuse of the enzyme. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the  
15 available literature. Alternatively, the same methods may be used to identify new substrates.

In addition, the protein of the invention or part thereof may be useful to identify or quantify the amount of a given substrate in biological fluids, foods, water, air, solutions and the like. In a preferred embodiment, the protein of the invention or part thereof is used in assays and diagnostic kits for the identification and quantification of exogenous substrates in bodily fluids including blood,  
20 lymph, saliva or other tissue samples, in addition to bacterial, fungal, plant, yeast, viral or mammalian cell cultures. In a preferred embodiment, the protein of the invention or part thereof is used to detect, identify, and or quantify eubacteria using reagents and assays described in US patent 5,935,804. Briefly, the protein of the invention or part thereof is catalytically inactivated, i.e. capable of binding but not cleaving a peptidoglycan comprising NAc-muramic acid in the eubacteria, using  
25 any of the methods known to those skilled in the art including those which produce a mutant enzyme, a recombinant-enzyme, or a chemically inactivated enzyme. The catalytically inactive protein of the invention is then incubated with an aliquot of a biological sample under conditions suitable for binding of the inactive enzyme to the peptidoglycan substrate. Then, the bound enzyme is detected to assess the presence or amount of the eubacteria in the biological sample.

30 In another embodiment, the nucleic acid of the invention or part thereof may be used to increase disease resistance of plants to bacterial, fungal and/or viral infections. A polynucleotide containing the nucleic acid of the invention or part thereof is introduced into the plant genome in conditions allowing correct expression of the transgenic protein using any methods known to those skilled in the art including those disclosed in US patents 5,349,122 and 5,850,025.

35 In another preferred embodiment, the protein of the invention or part thereof may be useful to treat and/or prevent bacterial, fungal and viral infections in humans or in animals caused by various agents including but not limited to *Streptococcus*, *Veillonella alcalescens*, *Actinomyces*,

Herpes simplex, *Candida albicans*, *Micrococcus lysodeikticus* and HIV by hydrolyzing the glycosylated compounds contained in such micro-organisms. In still a preferred embodiment, the protein of the invention or part thereof is used to prevent and/or treat bacterial, fungal and viral infections in immunocompromised individuals who lack fully functional immune systems, such as  
5 neonates or geriatric patients or HIV-infected individuals, or who suffer from a disease affecting the respiratory tract such as cystic fibrosis or the gastrointestinal tract such as ulcerative colitis or sprue.

In still another embodiment, the protein of the invention or part thereof may be used as a growth factor for in vitro cell culture, preferably for T cells and T cell lines, as described in US patent 5,468,635.

10 In addition, the protein of the invention or part thereof may be used to identify inhibitors for mechanistic and clinical applications. Such inhibitors may then be used to identify or quantify the protein of the invention in a sample, and to diagnose, treat or prevent any of the disorders where the protein's hydrolytic, immunostimulatory and/or inflammatory activities is/are undesirable and/or deleterious including but not limited to amyloidosis, colitis, lysosomal diseases, inflammatory and  
15 immune disorders including allergies and leukaemia. The protein of the invention may also be used to monitor host cell membranes for neoplastic transformation.

In still another embodiment, the invention relates to methods and compositions using the protein of the invention or part thereof as a marker protein to selectively identify tissues, preferably germ cells, more preferably testis. For example, the protein of the invention or part may be used to  
20 synthesize specific antibodies using any techniques known to those skilled in the art including those described therein. Such tissue-specific antibodies may then be used to identify tissues of unknown origin, for example, forensic samples, differentiated tumor tissue that has metastasized to foreign bodily sites, or to differentiate different tissue types in a tissue cross-section using immunochemistry.

25 Protein of SEQ ID NO:101 (internal designation 108-005-5-O-F9-FL)

The protein of SEQ ID NO:101 encoded by the extended cDNA SEQ ID NO: 51 shows homology with the *Drosophila* rhythmically expressed gene 2 protein (Genbank accession number U65492) and with a 2-haloalkanoic acid dehalogenase (Embl accession number AJ248288). In addition, the protein of SEQ ID NO:71 exhibits the pfam signature for haloacid dehalogenase-like  
30 hydrolase family from positions 7 to 214.

Expression of the mRNA coding for Dreg-2 is dependent on the interplay between light-dark cycle, feeding conditions and expression of the *per* gene which is essential to the function of the endogenous circadian pacemaker (Van Gelder *et al.*, *Curr. Biol.*, 5:1424-1436 (1995)). The matched pfam hydrolase family include proteins which are structurally different from the alpha/beta  
35 hydrolase family and which include L-2-haloacid dehalogenase, epoxide hydrolases and phosphatases (see Pfam accession number PF00702).

Organohalogen compounds are by-products in several industrial processes that are considered as environmental pollutants. The detection of trihalomethanes, halogenated acetic acids, halogenated acetonitriles and halogenated ketones in city water has become a great problem because of their liver toxicity and mutagenicity. Halogenated organic acids, for example halogenated acetic acids such as chloroacetic acid, dichloroacetic acid, trichloroacetic acid and bromoacetic acid have been designated as environment surveillance items in Japan since 1993. Increasing environmental concerns have created a demand for products that are free from such environmentally unsound byproducts. Physical methods of decontaminating aqueous reaction products containing unwanted nitrogen-free organohalogen byproducts are known, such as solvent extraction with a water-immiscible solvent, or adsorption on a solid adsorbent, such as charcoal. However, such known methods can result in depletion of the reaction product, as well as requiring costly measures to recover and purify the solvent or adsorbent. Furthermore, such methods still leave the problem of how to ultimately dispose of the contaminants such as undesired halogenated oxyalkylene compounds. As one of the countermeasures, for example, biodegradation treatment such as a bioreactor is very useful because treatment can be conducted under mild conditions and is relatively low in cost. The conversion of nitrogen-free organohalogen compounds with microorganisms containing a dehalogenase is also known. For example, C. E. Castro, et al. ("Biological Cleavage of Carbon-Halogen Bonds Metabolism of 3-Bromopropanol by *Pseudomonas* sp.", *Biochimica et Biophysica Acta*, 100, 384-392, 1965) describe the use of *Pseudomonas* sp. isolated from soil that metabolizes 3-bromopropanol in sequence to 3-bromopropionic acid, 3-hydroxypropionic acid and CO<sub>2</sub>. Various U.S. Patents also describe the use of microorganisms for dehalogenating halohydrins, e.g. U.S. Pat. Nos. 4,452,894; 4,477,570; and 4,493,895.

Epoxide hydrolases are a family of enzymes which hydrolyze a variety of exogenous and endogenous epoxides to their corresponding diols. Compounds containing the epoxide functionality have become common environmental contaminants because of their wide use as pesticides, sterilants, and industrial precursors. Such compounds also occur as products, by-products, or intermediates in normal metabolism and as the result of spontaneous oxidation of membrane lipids (i.e. see, Brash, et al., *Proc. Natl. Acad. Sci.*, 85:3382-3386 (1988), and Sevanian, A., et al., *Molecular Basis of Environmental Toxicology* (Bhatnager, R. S., ed.) pp. 213-228, Ann Arbor Science, Michigan (1980)). As three-membered cyclic ethers, epoxides are often very reactive and have been found to be cytotoxic, mutagenic and carcinogenic (i.e. see Sugiyama, S., et al., *Life Sci.* 40:225-231 (1987)). Cleavage of the ether bond in the presence of electrophiles often results in adduct formation. As a result, epoxides have been implicated as the proximate toxin or mutagen for a large number of xenobiotics. Reactions of detoxification using epoxide hydrolases typically decrease the hydrophobicity of a compound, resulting in a more polar and thereby excretable substance. In addition to degradation of potential toxic epoxides, dehalogenases are believed to play a role in the formation or degradation of endogenous chemical mediators (see US patent 5,445,956).

Many eukaryotic cell functions, including signal transduction, cell adhesion, gene transcription, RNA splicing, apoptosis and cell proliferation, are controlled by protein phosphorylation which is in turn regulated by the dynamic relationship between kinases and phosphatases (see US patent 6,040,323 for a short review). Thus, the protein phosphatases represent  
5 unique and attractive targets for small-molecule inhibition and pharmacological intervention. In addition, hydrolytic enzymes such as alkaline phosphatase are frequently used as markers or labels in enzyme-linked assays for biological molecules and other analytes of interest such as drugs, hormones, steroids and cancer markers.

It is believed that the protein of SEQ ID NO: 101 or part thereof is an hydrolase, preferably  
10 a phosphatase, an ether hydrolase or an hydrolase acting on C-halide bonds. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 101 from positions 7 to 214. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 101 having any of the biological activity described herein. The hydrolytic activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those  
15 described in US patents 5,445,942; 5,445,956, 6,017,746 and 5,871,616.

The invention relates to methods and compositions using the protein of the invention or part thereof to hydrolyze one or several substrates, alone or in combination with other substances, either in vitro or in vivo. Such substrates are compounds containing phosphoric ester bonds, ether bonds or C-halide bonds. For example, the protein of the invention or part thereof is added to a sample  
20 containing the substrate(s) in conditions allowing hydrolysis, and allowed to catalyze the hydrolysis of the substrate(s). In a preferred embodiment, the hydrolysis is carried out using any assay known to those skilled in the art including those described by the US patents 5,445,942; 5,445,956, 6,017,746 and 5,871,616. In a preferred embodiment, the protein of the invention is used to hydrolyze environmental pollutants, preferably organohalogen compounds and epoxide, such as  
25 those cited below using any of the methods and techniques described in US patents 6,017,746 and 5,871,616.

The invention relates to methods and compositions using the protein of the invention or part thereof to diagnose, prevent and/or treat several disorders of the circadian rhythm including, but not limited to, insomnia, depression, stress, night work or jet lag. For diagnostic purposes, the  
30 overexpression or the improper temporal expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals.

Protein of SEQ ID NO: 95 (internal designation 122-005-2-0-F11-FLC)

The protein of SEQ ID NO: 95 encoded by the cDNA of SEQ ID NO:45 exhibits homology  
35 with a fragment of NADH-cytochrome b5 reductases of rat, bovine and human species which are part of the mitochondrial electron transport chain (Genbank accession numbers J03867, M83104 and Y09501, respectively). This homology includes the flavin-adenine dinucleotide (FAD)-binding

domain of this family of proteins from positions 118 to 148, and 157 to 192. Moreover, the 3 lysine residues shown to be implicated in the formation of charged ion pairs with carboxyl groups on NADH-cytochrome b5 reductase during interactions between the active sites of cytochrome b5 and NADH-cytochrome b5 reductase are conserved in the protein of the invention at positions 46, 112 and 150 (Strittmatter, P. et al. (1990) J. Biol. Chem. 265: 21709-13). In addition, the protein of the invention exhibits motif signatures for cytochrome b5 reductase from positions 123 to 138, 163 to 180, and 256 to 265, motif signatures for eukaryotic molybdopterin oxidoreductases from positions 256 to 266 and 256 to 268, and motif signatures for flavoprotein pyridine nucleotide cytochrome reductases from positions 110 to 120, 163 to 177, and 163 to 179.

10 NADH-cytochrome b5 reductase proteins belong to a flavoenzyme family sharing common structural features and whose members (ferredoxin-NADP<sup>+</sup> reductase, NADPH-cytochrome P450 reductase, NADPH-sulfite reductase, NADH-cytochrome b5 reductase and NADH-nitrate reductase) are involved in photosynthesis, in the assimilation of nitrogen and sulfur, in fatty-acid oxidation, in the reduction of methemoglobin and in the metabolism of many pesticides, drugs and carcinogens  
15 (Karplus et al., Science, 251:60-6 (1991)). In addition, cytochrome b5 reductase is thought to play a role in the prevention of apoptosis following oxidative stress (see review by Villalba et al., Mol Aspects Med 18 Suppl:S7-13 (1997)).

It is believed that the protein of SEQ ID NO: 95 may be an oxidoreductase. Thus it may play a role in electron transport and general aerobic metabolism and may be associated with mitochondrial  
20 membranes. In addition, the protein of the invention may be able to use FAD and/or molybdopterin as cofactors. It may be involved in photosynthesis, in the assimilation of nitrogen and sulfur, in fatty-acid oxidation, in the reduction of methemoglobin and in the metabolism of many pesticides, drugs and carcinogens. Preferred polypeptides of the SEQ ID NO: 95 from positions 118 to 148, 157 to 192, 123 to 138, 163 to 180, 256 to 265, 256 to 266, 256 to 268, 110 to 120, 163 to 177, and 163 to 179. Other  
25 preferred polypeptides of the invention are fragments of SEQ ID NO: 95 having any of the biological activity described herein. The oxidoreductase activity of the protein of the invention may be assayed using any technique known to those skilled in the art. The ability to bind a cofactor may also be assayed using any techniques well known to those skilled in the art including, for example, the assay for binding NAD described in US patent 5,986,172.

30 An object of the present invention are compositions and methods of targeting heterologous compounds, either polypeptides or polynucleotides to mitochondria by recombinantly or chemically fusing a fragment of the protein of the invention to an heterologous polypeptide or polynucleotide. Preferred fragments are signal peptide, amphiphilic alpha helices and/or any other fragments of the protein of the invention, or part thereof, that may contain targeting signals for mitochondria  
35 including but not limited to matrix targeting signals as defined in Herrman and Neupert, Curr. Opinion Microbiol. 3:210-4 (2000); Bhagwat et al. J. Biol. Chem. 274:24014-22 (1999), Murphy Trends Biotechnol. 15:326-30 (1997); Glaser et al. Plant Mol Biol 38:311-38 (1998); Ciminale et al.



Oncogene 18:4505-14 (1999). Such heterologous compounds may be used to modulate mitochondria's activities. For example, they may be used to induce and/or prevent mitochondrial-induced apoptosis or necrosis. In addition, heterologous polynucleotides may be used for mitochondrial gene therapy to replace a defective mitochondrial gene and/or to inhibit the deleterious expression of a mitochondrial gene.

In another embodiment, the protein of the invention or part thereof is used to prevent cells to undergo apoptosis. In a preferred embodiment, the apoptosis active polypeptide is added to an in vitro culture of mammalian cells in an amount effective to reduce apoptosis. Furthermore, the protein of the invention or part thereof may be useful in the diagnosis, the treatment and/or the prevention of disorders in which apoptosis is deleterious, including but not limited to immune deficiency syndromes (including AIDS), type I diabetes, pathogenic infections, cardiovascular and neurological injury, alopecia, aging, degenerative diseases such as Alzheimer's Disease, Parkinson's Disease, Huntington's disease, dystonia, Leber's hereditary optic neuropathy, schizophrenia, and myodegenerative disorders such as "mitochondrial encephalopathy, lactic acidosis, and stroke" (MELAS), and "myoclonic epilepsy ragged red fiber syndrome" (MERRF).

The invention further relates to methods and compositions using the protein of the invention or part thereof to diagnose, prevent and/or treat several disorders in which energy metabolism is impaired, or needs to be impaired, including but not limited to mitochondriocytopathies, necrosis, aging, neurodegenerative diseases, myopathies, methemoglobinemia, hyperlipidemia, obesity, cardiovascular disorders and cancer. For diagnostic purposes, the expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals. For prevention and/or treatment purposes, the protein of the invention may be used to enhance electron transport and increase energy delivery using any of the gene therapy methods described herein.

**25 Protein of SEQ ID NO: 113 (internal designation 108-014-5-0-C7-FLC)**

The protein of SEQ ID NO: 113 encoded by the extended cDNA SEQ ID NO: 63 shows homology with a fragment of a cold active protease isolated from *Flavobacterium balustinum* (Genseq accession number W23332) which degrades casein, gelatin, haemoglobin and albumin. This protease is able to degrade proteins at low temperatures or in presence of organic solvents that are volatile at normal processing temperature.

These data suggest that the protein of SEQ ID NO: 113 or part thereof is an hydrolase, preferably a protease. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 113 from positions 1 to 44. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 113 having any of the biological activity described herein. The hydrolytic activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in US patent 6,069,229.

The invention relates to methods and compositions using the protein of the invention or part thereof to hydrolyze one or several substrates, alone or in combination with other substances. Such substrates are compounds containing peptide bonds. For example, the protein of the invention or part thereof is added to a sample containing the substrate(s) in conditions allowing hydrolysis, and  
5 allowed to catalyze the hydrolysis of the substrate(s). In a preferred embodiment, the hydrolysis is carried out using a standard assay such as those described by the US patent 6,069,229.

In a preferred embodiment, compositions comprising the protein of the present invention or part thereof are added to samples as a "cocktail" with other hydrolytic enzymes such as those described in US patents 5,458,876 and 5,041,326. The advantage of using a cocktail of hydrolytic enzymes is that  
10 one is able to hydrolyze a wide range of substrates without knowing the specificity of any of the enzymes. Using a cocktail of hydrolytic enzymes also protects a sample from a wide range of future unknown protein contaminants from a vast number of sources. For example, the protein of the invention or part thereof is added to samples where contaminating substrates is undesirable. For example, the protein of the invention or part thereof may be used to remove protein contaminants from  
15 nucleic acid preparations, to remove cells from cultureware. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other hydrolytic enzymes, using techniques well known in the art, to form an affinity chromatography column. A sample containing the undesirable substrate is run through the column to remove the substrate. Immobilizing the protein of the invention or part thereof on a support is particularly  
20 advantageous for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the enzyme from the batch of product and subsequent reuse of the enzyme. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature.  
25 Alternatively, the same methods may be used to identify new substrates.

The protease of the invention may be used in many industrial processes, including in detergents and cleaning products, e.g., to degrade protein materials such as blood and stains or to clean contact lenses, in leather production, e.g., to remove hair, in baking, e.g., to break down glutens, in flavorings, e.g., soy sauce, in meat tenderizing, e.g., to break down collagen, in gelatin or  
30 food supplement production, in the textile industry, in waste treatment, and in the photographic industry. See, e.g., Gusek (1991) Inform 1:14-18; Zamost, et al. (1996) J. Industrial Microbiol. 8:71-82; James and Simpson (1996) CRC Critical Reviews in Food Science and Nutrition 36:437-463; Teichgraeber, et al. (1993) Trends in Food Science and Technology 4:145-149; Tjwan, et al. (1993) J. Dairy Research 60:269-286; Haard (1992) J. Aquatic Food Product Technology 1:17-35; van Dijk  
35 (1995) Laundry and Cleaning News 21:32-33; Nolte, et al. (1996) J. Textile Institute 87:212-226; Chikkodi, et al. (1995) Textile Res. J. 65:564-569; and Shih (1993) Poultry Science 72:1617-1620; PCT publication WO9925848-A1.

In addition, the protein of the invention or part thereof may be used to identify inhibitors for mechanistic and clinical applications. Such inhibitors may then be used to identify or quantify the protein of the invention in a sample, and to diagnose, treat or prevent any of the disorders where the protein's hydrolytic activity is undesirable and/or deleterious such as disorders characterized by  
5 tissue degradation including but not limited to amyloidosis, colitis, lysosomal diseases, arthritis, muscular dystrophy, inflammation, tumor invasion, glomerulonephritis, parasite-borne infections, Alzheimer's disease, periodontal disease, and cancer metastasis.

Protein of SEQ ID NO: 81 (internal designation 116-047-3-0-B1-FLC)

The protein of SEQ ID NO: 81 encoded by the extended cDNA SEQ ID NO: 31 shows  
10 homology with the ribokinase rbsk (Embl accession number Q9X4M5) which is part of the pfkb family of kinases. In addition, the protein of the invention exhibits the pfam signature for this family of carbohydrate and purine kinases from positions 28 to 94.

The pfkb family of carbohydrate kinase is composed of evolutionary related kinases including fructokinases, ribokinase, adenosine kinase, inosine-guanosine kinase, and  
15 phosphotagatokinase (for a short review see Prosite entry N°PD0C00504).

It is believed that the protein of SEQ ID NO: 81 or part thereof is a carbohydrate or purine kinase. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 81 from positions 28 to 94, and from 1 to 94. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 81 having any of the biological activity described herein. The kinase  
20 activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described by the US patents 5,756,315 and 5,861,294.

The invention relates to methods and compositions using the protein of the invention or part thereof to phosphorylate substrates, preferably carbohydrate or purine substrates. For example, the protein of the invention or part thereof is added to a sample containing the substrate(s) as well as a  
25 phosphate donor group in conditions allowing the transfer of the phosphorus group, and allowed to transfer the phosphorus group to the substrate(s). In a preferred embodiment, the kination is carried out using a standard assay including those described by the US patents 5,756,315 and 5,861,294. Such phosphorylated purine substrates, such as 5'-IMP and 5'-GMP, have an enhanced flavor activity and may be used as seasoning agents.

30 In another embodiment, the present invention relates to processes and compositions for controlling the production of phosphorylated substrates, preferably carbohydrate and purine substrates, more preferably glucose, fructose, inosine, guanosine, adenosine, wherein a cell or an organism is an organism is genetically engineered either to produce the protein of the invention or part thereof or to inhibit the endogenous expression of the protein of the invention or part thereof  
35 using methods and techniques known to those skilled in the art including those described in US patent 6,031,154. For example, a plant may be genetically engineered to express the protein of the invention or part thereof, thereby increasing the amount of phosphorylated carbohydrate substrates

to be imported into plastids and ultimately enhancing starch biosynthesis. On the contrary, a fruit may also be genetically engineered to inhibit the endogenous expression of the protein of the invention in order to increase the concentration of non phosphorylated carbohydrates, ultimately leading to fruits with enhanced sweetness.

- 5       The invention further relates to methods and composition using the protein of the invention or part thereof to diagnose, prevent and/or treat disorders in which the availability of phosphorylated substrates, preferably carbohydrate and purine substrates, is impaired or needs to be impaired. In a preferred embodiment, the protein of the invention or part thereof may be used to activate pharmacologically active nucleosides including but not limited to tubercidin, formycin, ribavirin,
- 10 pyrazofurin and 6-(methylmercapto)purine riboside which are antimetabolites with cytotoxic, anticancer and antiviral properties. In another preferred embodiment, the protein of the invention or part thereof may be used to compensate alterations observed in endogenous adenosine kinase activity observed in certain disorders including but not limited to hepatoma, hepatectomy, gout, and HIV infection. In still another preferred embodiment, the protein of the invention or part thereof may be used to modulate the
- 15 concentration of adenosine which was shown to play important physiological roles. In the central nervous system, adenosine inhibits the release of certain neurotransmitters (Corradetti et al., *Eur. J. Pharmacol.* 1984, 104: 19-26), stabilizes membrane potential (Rudolphi et al., *Cerebrovasc. Brain Metab. Rev.* 1992, 4: 346-360), functions as an endogenous anticonvulsant (Dragunow, *Trends Pharmacol. Sci.* 1986, 7:128-130) and may have a role as an endogenous neuroprotective agent
- 20 (Rudolphi et al., *Trends Pharmacol. Sci.* 1992, 13: 439-445). Adenosine has also been implicated in modulating transmission in pain pathways in the spinal cord (Sawynok et al., *Br. J. Pharmacol.* 1986, 88: 923-930), and in mediating the analgesic effects of morphine (Sweeney et al., *J. Pharmacol. Exp. Ther.* 1987, 243: 657-665). In the immune system, adenosine inhibits certain neutrophil functions and exhibits anti-inflammatory effects (Cronstein, *J. Appl. Physiol.* 1994, 76: 5-13). Adenosine also exerts a
- 25 variety of effects on the cardiovascular system, including vasodilation, impairment of atrioventricular conduction and endogenous cardioprotection in myocardial ischemia and reperfusion (Mullane and Williams, in *Adenosine and Adenosine Receptors* 1990 (Williams, ed) Humana Press, New Jersey, pp. 289-334). The widespread actions of adenosine also include effects on the renal, respiratory, gastrointestinal and reproductive systems, as well as on blood cells and adipocytes. Endogenous
- 30 adenosine release appears to have a role as a natural defense mechanism in various pathophysiologic conditions, including cerebral and myocardial ischemia, seizures, pain, inflammation and sepsis. While adenosine is normally present at low levels in the extracellular space, its release is locally enhanced at the site(s) of excessive cellular activity, trauma or metabolic stress. Once in the extracellular space, adenosine activates specific extracellular receptors to elicit a variety of responses which tend to restore
- 35 cellular function towards normal (Bruns, *Nucleosides Nucleotides*, 1991, 10: 931-943; Miller and Hsu, *J. Neurotrauma*, 1992, 9: S563-S577). Adenosine has a half-life measured in seconds in extracellular fluids (Moser et al., *Am. J. Physiol.* 1989, 25: C799-C806), and its endogenous actions are therefore

highly localized. The inhibition of adenosine kinase can result in augmentation of the local adenosine concentrations at foci of tissue injury, further enhancing cytoprotection. This effect is likely to be most pronounced at tissue sites where trauma results in increased adenosine production, thereby minimizing systemic toxicities. Pharmacological compounds directed towards adenosine kinase inhibition provide  
5 potential effective new therapies for disorders benefited by the site- and event-specific potentiation of adenosine.

Protein of SEQ ID NO: 107 (internal designation 108-011-5-O-C7-FLC)

The protein of SEQ ID NO: 107 encoded by the extended cDNA SEQ ID NO: 57 shows homology with the chicken ribonuclease A (Embl accession number X61192) which is part of the  
10 pancreatic ribonuclease family. In addition, the protein of the invention exhibits the pfam signature for this family of pancreatic ribonucleases from positions 17 to 67.

Ribonucleases are proteins which catalyze the hydrolysis of phosphodiester bonds in RNA chains. Pancreatic ribonucleases are pyrimidic-specific ribonucleases present in high quantity in the pancreas of a number of mammalia taxa and of a few reptiles. In addition to their function in  
15 hydrolysis of RNA, ribonucleases have evolved to support a variety of other physiological activities. Such activities include anti-parasite, anti-bacterium, anti-virus, anti-neoplastic activities, neurotoxicity, and angiogenesis. For example, bovine seminal ribonuclease is anti-neoplastic (Laceetti, P. et al. (1992) Cancer Res. 52: 4582-4586). Some frog ribonucleases display both anti-viral and anti-neoplastic activity (Youle, R. J. et al. (1994) Proc. Natl. Acad. Sci. USA 91: 6012-  
20 6016; Mikulski, S. M. et al. (1990) J. Natl. Cancer Inst. 82: 151-152; and Wu, Y. -N. et al. (1993) J. Biol. Chem. 268: 10686-10693). Angiogenin is a tRNA-specific ribonuclease which binds actin on the surface of endothelial cells for endocytosis. Endocytosed angiogenin is translocated to the nucleus where it promotes endothelial invasiveness required for blood vessel formation (Moroianu, J. and Riordan, J. F. (1994) Proc. Natl. Acad. Sci. USA 91: 1217-1221). Eosinophil-derived  
25 neurotoxin (EDN) and eosinophil cationic protein (ECP) are related ribonucleases which possess neurotoxicity (Beintema, J. J. et al. (1988) Biochemistry 27: 4530-4538; Ackerman, S. J. (1993) In Makino, S. and Fukuda, T., Eosinophils: Biological and Clinical Aspects. CRC Press, Boca Raton, Fla., pp 33-74). In addition, ECP exhibits cytotoxic, anti-parasitic, and anti-bacterial activities. A EDN-related ribonuclease, named RNase k6, is shown to express in normal human monocytes and  
30 neutrophils, suggesting a role for this ribonuclease in host defense (Rosenberg, H. F. and Dyer, K. D. (1996) Nuc. Acid. Res. 24: 3507-3513).

It is believed that the protein of SEQ ID NO: 107 or part thereof is a ribonuclease. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 107 from positions 17 to 67. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 107  
35 having any of the biological activity described herein. The ribonuclease activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in US patent 5,866,119.

The invention relates to methods and compositions using the protein of the invention or part thereof to hydrolyze one or several substrates, preferably nucleic acids, more preferably RNA, alone or in combination with other substances. For example, the protein of the invention or part thereof is added to a sample containing the substrate(s) in conditions allowing hydrolysis, and allowed to  
5 catalyze the hydrolysis of the substrate(s).

In a preferred embodiment, the protein of the invention or part thereof may be used to remove contaminating RNA in a biological sample, alone or in combination with other nucleases. In a more preferred embodiment, the protein of the invention or part thereof may be used to purify DNA preparations from contaminating RNA, to remove RNA templates prior to second strand  
10 synthesis and prior to analysis of in vitro translation products. Compositions comprising the protein of the present invention or part thereof are added to biological samples as a "cocktail" with other nucleases. The advantage of using a cocktail of hydrolytic enzymes is that one is able to hydrolyze a wide range of substrates without knowing the specificity of any of the enzymes. Such cocktails of nucleases are commonly used in molecular biology assays, for example to remove unbound RNA in  
15 RNase protection assays. Using a cocktail of hydrolytic enzymes also protects a sample from a wide range of future unknown RNA contaminants from a vast number of sources. For example, the protein of the invention or part thereof is added to samples where contaminating substrates is undesirable. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other hydrolytic enzymes, using  
20 techniques well known in the art, to form an affinity chromatography column. A sample containing the undesirable substrate is run through the column to remove the substrate. Immobilizing the protein of the invention or part thereof on a support is particularly advantageous for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the enzyme from the batch of product and subsequent reuse of the enzyme.  
25 Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature. Alternatively, the same methods may be used to identify new substrates.

In another embodiment, the protein of the invention or part thereof may be used to  
30 decontaminate or disinfect samples infected by undesirable parasite, bacteria and/or viruses using any of the methods known to those skilled in the art including those described in Youle et al, (1994), supra; Mikulski et al (1990) supra, Wu et al (1993) supra.

In another embodiment, the present invention relates to compositions and methods using the protein of the invention or part thereof to selectively kill cells. The protein of the invention or part  
35 thereof is linked to a recognition moiety capable of binding to a chosen cell, such as lectins, receptors or antibodies thus generating cytotoxic reagents using methods and techniques described in US patent 5,955,073.

In another embodiment, the protein of the invention or part thereof may be used in the diagnosis, prevention and/or treatment of disorders associated with excessive cell proliferation such as cancer.

Protein of SEQ ID NO: 77 (internal designation 105-118-4-O-E6-FLC)

- 5       The protein of SEQ ID NO: 77 encoded by the extended cDNA SEQ ID NO: 27 is homologous to a hepatocellular carcinoma associated ring finger protein (Embl accession number AF247565) and homology with a putative anaphase-promoting complex subunit from *Drosophila* (Embl accession number AJ251510). In addition, the protein of the invention exhibits the pfam PHD zinc finger signature from positions 33 to 79.
- 10       Zinc finger domains are found in numerous zinc binding proteins which are involved in protein-nucleic acid interactions. They are independently folded zinc-containing mini-domains which are used in a modular repeating fashion to achieve sequence-specific recognition of DNA (Klug 1993 Gene 135, 83-92). Such zinc binding proteins are commonly involved in the regulation of gene expression, and usually serve as transcription factors (see US patents 5,866,325; 6,013,453
- 15       and 5,861,495). PHD fingers are  $C_4H_2$  zinc fingers spanning approximately 50-80 residues and distinct from RING fingers or LIM domains. They are thought to be mostly DNA or RNA binding domain but may also be involved in protein-protein interactions (for a review see Aasland et al, Trends Biochem Sci 20:56-59 (1995)).

- It is believed that the protein of SEQ ID NO: 77 or part thereof is a zinc binding protein,
- 20       preferably able to bind nucleic acids, more preferably a transcription factor. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 77 from positions 33 to 79. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 77 having any of the biological activity described herein. The nucleic acid binding activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art
- 25       including those described in US patent 6,013,453.

- The invention relates to methods and compositions using the protein of the invention or part thereof to bind to nucleic acids, preferably DNA, alone or in combination with other substances. For example, the protein of the invention or part thereof is added to a sample containing nucleic acid in conditions allowing binding, and allowed to bind to nucleic acids. In a preferred embodiment, the
- 30       protein of the invention or part thereof may be used to purify nucleic acids such as restriction fragments. In another preferred embodiment, the protein of the invention or part thereof may be used to visualize nucleic acids when the polypeptide is linked to an appropriate fusion partner, or is detected by probing with an antibody. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other DNA binding proteins, using
- 35       techniques well known in the art, to form an affinity chromatography column. A sample containing nucleic acids to purify is run through the column. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly for those embodiments in which the method is to be

practiced on a commercial scale. This immobilization facilitates the removal of the protein from the batch of product and subsequent reuse of the protein. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are  
5 described in the available literature.

In another embodiment, the present invention relates to compositions and methods using the protein of the invention or part thereof, especially the zinc binding domain, to alter the expression of genes of interest in a target cells. Such genes of interest may be disease related genes, such as oncogenes or exogenous genes from pathogens, such as bacteria or viruses using any techniques  
10 known to those skilled in the art including those described in US patents 5,861,495; 5,866,325 and 6,013,453.

In still another embodiment, the protein of the invention or part thereof may be used to diagnose, treat and/or prevent disorders linked to dysregulation of gene transcription such as cancer and other disorders relating to abnormal cellular differentiation, proliferation, or degeneration,  
15 including hyperaldosteronism, hypocortisolism (Addison's disease), hyperthyroidism (Grave's disease), hypothyroidism, colorectal polyps, gastritis, gastric and duodenal ulcers, ulcerative colitis, and Crohn's disease.

Protein of SEQ ID NO: 114 (internal designation 108-014-5-O-D12-FLC)

The protein of SEQ ID NO: 114 encoded by the extended cDNA SEQ ID NO: 64 shows  
20 homology with zinc binding proteins (Embl accession number Q9QZQ6 and Genseq accession number W69602). In addition, the protein of the invention exhibits the pfam RING zinc finger signature from positions 258 to 298.

Zinc binding (ZB) domains are found in numerous proteins which are involved in protein-nucleic acid or protein-protein interactions. ZB proteins are commonly involved in the regulation of  
25 gene expression, and may serve as transcription factors and signal transduction molecules. A ZB domain is generally composed of 25 to 30 amino acid residues which form one or more tetrahedral ion binding sites. The binding sites contain four ligands consisting of the sidechains of cysteine, histidine and occasionally aspartate or glutamate. The binding of zinc allows the relatively short stretches of polypeptide to fold into defined structural units which are well-suited to participate in  
30 macromolecular interactions (Berg, J. M. et al. (1996) Science 271:1081-1085). Zinc binding domains which contain a C<sub>3</sub>HC<sub>4</sub> sequence motif are known as RING domains (Lovering, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:2112-2116). The RING domain consists of eight metal binding residues, and the sequences that bind the two metal ions overlap (Barlow, P. N. et al. (1994) J. Mol. Biol. 237:201-211). Functions of RING finger proteins are mediated through DNA binding  
35 and include the regulation of gene expression, DNA recombination, and DNA repair (see Borden and Freemont, Curr Opin Struct Biol 6:395-401 (1996) and US patent 5,861,495).



It is believed that the protein of SEQ ID NO: 114 or part thereof is a zinc binding protein, preferably able to bind nucleic acids or proteins, more preferably a transcription factor. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 114 from positions 258 to 298. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 114 having any of the biological activity described herein. The nucleic acid binding activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in US patent 6,013,453.

The invention relates to methods and compositions using the protein of the invention or part thereof to bind to nucleic acids, preferably DNA, alone or in combination with other substances. For example, the protein of the invention or part thereof is added to a sample containing nucleic acid in conditions allowing binding, and allowed to bind to nucleic acids. In a preferred embodiment, the protein of the invention or part thereof may be used to purify nucleic acids such as restriction fragments. In another preferred embodiment, the protein of the invention or part thereof may be used to visualize nucleic acids when the polypeptide is linked to an appropriate fusion partner, or is detected by probing with an antibody. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other DNA binding proteins, using techniques well known in the art, to form an affinity chromatography column. A sample containing nucleic acids to purify is run through the column. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the protein from the batch of product and subsequent reuse of the protein. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature.

In another embodiment, the present invention relates to compositions and methods using the protein of the invention or part thereof, especially the zinc binding domain, to alter the expression of genes of interest in a target cells. Such genes of interest may be disease related genes, such as oncogenes or exogenous genes from pathogens, such as bacteria or viruses using any techniques known to those skilled in the art including those described in US patents 5,861,495; 5,866,325 and 6,013,453.

In still another embodiment, the protein of the invention or part thereof may be used to diagnose, treat and/or prevent disorders linked to dysregulation of gene transcription such as cancer and other disorders relating to abnormal cellular differentiation, proliferation, or degeneration, including hyperaldosteronism, hypocortisolism (Addison's disease), hyperthyroidism (Grave's disease), hypothyroidism, colorectal polyps, gastritis, gastric and duodenal ulcers, ulcerative colitis, and Crohn's disease.

Protein of SEQ ID NO: 105 (internal designation 108-008-5-O-G5-FLC)

The protein of SEQ ID NO: 105 encoded by the extended cDNA SEQ ID NO: 55 shows homology with zinc binding proteins (Embl accession number Q9VZJ9). In addition, the protein of the invention exhibits the pfam RING zinc finger signature from positions 302 to 339.

5 Zinc binding (ZB) domains are found in numerous proteins which are involved in protein-nucleic acid or protein-protein interactions. ZB proteins are commonly involved in the regulation of gene expression, and may serve as transcription factors and signal transduction molecules. A ZB domain is generally composed of 25 to 30 amino acid residues which form one or more tetrahedral ion binding sites. The binding sites contain four ligands consisting of the sidechains of cysteine,  
10 histidine and occasionally aspartate or glutamate. The binding of zinc allows the relatively short stretches of polypeptide to fold into defined structural units which are well-suited to participate in macromolecular interactions (Berg, J. M. et al. (1996) Science 271:1081-1085). Zinc binding domains which contain a  $C_3HC_4$  sequence motif are known as RING domains (Lovering, R. et al. (1993) Proc. Natl. Acad. Sci. USA 90:2112-2116). The RING domain consists of eight metal  
15 binding residues, and the sequences that bind the two metal ions overlap (Barlow, P. N. et al. (1994) J. Mol. Biol. 237:201-211). Functions of RING finger proteins are mediated through DNA binding and include the regulation of gene expression, DNA recombination, and DNA repair (see Borden and Freemont, Curr Opin Struct Biol 6:395-401 (1996) and US patent 5,861,495).

It is believed that the protein of SEQ ID NO: 105 or part thereof is a zinc binding protein,  
20 preferably able to bind nucleic acids or proteins, more preferably a transcription factor. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO: 105 from positions 302 to 339. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 105 having any of the biological activity described herein. The nucleic acid binding activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art  
25 including those described in US patent 6,013,453.

The invention relates to methods and compositions using the protein of the invention or part thereof to bind to nucleic acids, preferably DNA, alone or in combination with other substances. For example, the protein of the invention or part thereof is added to a sample containing nucleic acid in conditions allowing binding, and allowed to bind to nucleic acids. In a preferred embodiment, the  
30 protein of the invention or part thereof may be used to purify nucleic acids such as restriction fragments. In another preferred embodiment, the protein of the invention or part thereof may be used to visualize nucleic acids when the polypeptide is linked to an appropriate fusion partner, or is detected by probing with an antibody. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other DNA binding proteins, using  
35 techniques well known in the art, to form an affinity chromatography column. A sample containing nucleic acids to purify is run through the column. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly for those embodiments in which the method is to be

practiced on a commercial scale. This immobilization facilitates the removal of the protein from the batch of product and subsequent reuse of the protein. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are  
5 described in the available literature.

In another embodiment, the present invention relates to compositions and methods using the protein of the invention or part thereof, especially the zinc binding domain, to alter the expression of genes of interest in a target cells. Such genes of interest may be disease related genes, such as oncogenes or exogenous genes from pathogens, such as bacteria or viruses using any techniques  
10 known to those skilled in the art including those described in US patents 5,861,495; 5,866,325 and 6,013,453.

In still another embodiment, the protein of the invention or part thereof may be used to diagnose, treat and/or prevent disorders linked to dysregulation of gene transcription such as cancer and other disorders relating to abnormal cellular differentiation, proliferation, or degeneration,  
15 including hyperaldosteronism, hypocortisolism (Addison's disease), hyperthyroidism (Grave's disease), hypothyroidism, colorectal polyps, gastritis, gastric and duodenal ulcers, ulcerative colitis, and Crohn's disease.

Protein of SEQ ID NO: 106 (internal designation 108-011-5-O-B12-FL)

The protein of SEQ ID NO: 106 encoded by the extended cDNA SEQ ID NO: 56 shows  
20 homology to the predicted extracellular domain and part of transmembrane domain of interleukin-17 receptor of both human and murine species (Genbank accession numbers W04185 and W04184). These IL-17R proteins are thought to belong to a new family of receptors for cytokines which induce T cell proliferation, I-CAM expression and preferential maturation of haematopoietic precursors into neutrophils (Yao *et al.*, *Cytokine.*, 9:794-8001 (1997)). It is also thought to play a proinflammatory  
25 role and to induce nitric oxide. The protein of the invention has a 21 amino acid transmembrane domain (positions 172 to 192) as predicted by the software TopPred II (Claros and von Heijne, *CABIOS applic. Notes*, 10 :685-686 (1994)) matching the 21 amino acid putative transmembrane domain of human interleukin-17 receptor.

It is believed that the protein of SEQ ID NO: 106 plays a role in regulating immune and/or  
30 inflammatory responses. Preferred polypeptides of the invention are fragments of SEQ ID NO: 106 having any of the biological activities described herein.

The present invention relates to methods and compositions using the protein of the invention or part thereof to inhibit the proliferation and/or the differentiation of lymphocytes or lymphocytic cell lines, both in vitro and in vivo. For example, soluble forms of the protein of the invention or  
35 part thereof may be added to cell culture medium in an amount effective to inhibit the proliferation and/or the differentiation of lymphocytes and/or lymphocytic cell lines.

Another embodiment relates to methods and compositions using the protein of the invention or part thereof to diagnose, treat and/or prevent several disorders including, but not limited to, cancer, inflammatory and immune disorders, septic shock and impotence. Immune and inflammatory disorders include Addison's disease, AIDS, acute or chronic inflammation due to antigen, antibody  
5 and/or complement deposition, acute and delayed hypersensitivity, adult respiratory distress syndrome, allergies, anemia, arthritis, asthma, atherosclerosis, bronchitis, cholangitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, encephalitis, endocarditis, atrophic gastritis, glomerulonephritis, gout, graft rejection, graft-versus-host disease, Graves' disease, hepatitis, hyper eosinophilia, irritable bowel syndrome,  
10 lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polycystic kidney disease, polymyositis, reperfusion injury, rheumatoid arthritis, scleroderma, Sjogren's syndrome, and autoimmune thyroiditis.

In addition, this protein may also be useful to modulate immune and/or inflammatory responses to infectious responses and/or to suppress graft rejection. For example, soluble forms of  
15 the protein of the invention or blocking antibodies, or antagonists may be used to inhibit and/or reduce immune and/or inflammatory responses.

Protein of SEQ ID NO: 97 (internal designation 108-004-5-O-B12-FLC)

The protein of SEQ ID NO: 97 encoded by the extended cDNA SEQ ID NO: 47 is homologous to a human protein either described as a maid-like gene (Embl accession number  
20 AF132000) or a human secreted protein (Geneseq accession number Y41330).

Maid is a maternally transcribed gene encoding a putative regulator of basic helix-loop-helix transcription factor in the mouse egg and zygote. In vitro, maid is able to bind to DNA. When transfected, maid reduces the transcription of a CAT-reporter regulated by an E12/MyoD enhancer (Hwang et al, Dev Dyn, 209:217-26 (1997)).

25 It is believed that the protein of SEQ ID NO: 97 or part thereof is involved in the regulation of gene transcription, probably through direct binding to DNA. Preferred polypeptides of the invention are fragments of SEQ ID NO: 97 having any of the biological activity described herein. The nucleic acid binding activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in US patent 6,013,453.

30 The invention relates to methods and compositions using the protein of the invention or part thereof to bind to nucleic acids, preferably DNA, alone or in combination with other substances. For example, the protein of the invention or part thereof is added to a sample containing nucleic acid in conditions allowing binding, and allowed to bind to nucleic acids. In a preferred embodiment, the protein of the invention or part thereof may be used to purify nucleic acids such as restriction  
35 fragments. In another preferred embodiment, the protein of the invention or part thereof may be used to visualize nucleic acids when the polypeptide is linked to an appropriate fusion partner, or is detected by probing with an antibody. Alternatively, the protein of the invention or part thereof may

be bound to a chromatographic support, either alone or in combination with other DNA binding proteins, using techniques well known in the art, to form an affinity chromatography column. A sample containing nucleic acids to purify is run through the column. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the protein from the batch of product and subsequent reuse of the protein. Immobilizing the protein of the invention or part thereof on a support advantageous is particularly for those embodiments in which the method is to be practiced on a commercial scale. This immobilization facilitates the removal of the protein from the batch of product and subsequent reuse of the protein. Immobilization of the protein of the invention or part thereof can be accomplished, for example, by inserting a cellulose-binding domain in the protein. One of skill in the art will understand that other methods of immobilization could also be used and are described in the available literature.

In another embodiment, the present invention relates to compositions and methods using the protein of the invention or part thereof to alter the expression of genes of interest in a target cell. Such genes of interest may be disease related genes, such as oncogenes or exogenous genes from pathogens, such as bacteria or viruses using any techniques known to those skilled in the art including those described in US patents 5,861,495; 5,866,325 and 6,013,453.

In still another embodiment, the protein of the invention or part thereof may be used to diagnose, treat and/or prevent disorders linked to dysregulation of gene transcription such as cancer and other disorders relating to abnormal cellular differentiation, proliferation, or degeneration, including hyperaldosteronism, hypocortisolism (Addison's disease), hyperthyroidism (Grave's disease), hypothyroidism, colorectal polyps, gastritis, gastric and duodenal ulcers, ulcerative colitis, and Crohn's disease.

Protein of SEQ ID NO: 122 (internal designation 108-020-5-O-D4-FLC)

The protein of SEQ ID NO: 122 encoded by the extended cDNA SEQ ID NO: 72 shows homology to a murine transmembrane protein (Genbank accession number BAA92746). When expressed in E. Coli, the matched which suppresses bacterial growth (Inoue et al, Biochem Biophys Res Commun 268:553-61 (2000)). In addition, a transmembrane domain is predicted for the protein of SEQ ID NO: 122 from positions 36 to 56 by the software TopPred II (Claros and von Heijne, CABIOS applic. Notes, 10 :685-686 (1994)).

It is believed that the protein of SEQ ID NO: 122 or part thereof is able to suppress bacterial growth. Preferred polypeptides of the invention are fragments of SEQ ID NO: 97 having any of the biological activity described herein. The growth inhibiting activity of the protein of the invention or part thereof may be assayed using any of the assays known to those skilled in the art including those described in Inoue et al, supra.

The invention relates to methods and compositions using the protein of the invention or part thereof to suppress bacterial growth. For example, the protein of the invention may be expressed in

a bacteria, preferably E. coli, using recombinant DNA technology methods known to those skilled in the art. The bacterial growth may then be assessed using any methods or techniques known to those skilled in the art.

Protein of SEQ ID NO: 96 (internal designation 122-007-3-O-D10-FLC)

5       The protein of SEQ ID NO: 96 encoded by the extended cDNA SEQ ID NO: 46 shows homology to a human secreted protein highly expressed in testis (Genseq accession number Y06940). In addition, it exhibits an emotif signature for the flagellar biosynthetic protein fliR family from positions 7 to 27.

10       FliR is an integral membrane protein located in the flagellar basal body and thought to be a component of the type III export apparatus (Fan et al, Mol Microbiol 26:1035-46 (1997)).

      It is believed that the protein of SEQ ID NO: 96 or part thereof plays a role in gametogenesis, maybe as a component of spermatozooids. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO:96 from positions 7 to 27. Other preferred polypeptides of the invention are fragments of SEQ ID NO: 96 having any of the biological activity  
15       described herein.

      The invention relates to methods and compositions using the protein of the invention or part thereof to diagnose, treat and/or prevent fertility disorders. For diagnostic purposes, the expression of the protein of the invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals.  
20       For prevention and/or treatment purposes, the protein of the invention may be used to enhance gametogenesis using any of the gene therapy methods described herein or known to those skilled in the art.

      Moreover, antibodies to the protein of the invention or part thereof may be used for detection of gametes using any techniques known to those skilled in the art.

25       Protein of SEQ ID NO: 110 (internal designation 108-013-5-0-G5-FLC)

      The protein of SEQ ID NO: 110 encoded by the extended cDNA SEQ ID NO: 60 displays the pfam signature for the N-terminus of the alpha-macroglobulin A2M family from positions 17 to 40. A2M-like proteins are able to inhibit all four classes of proteinases by a "trapping mechanism" (see Prosite entry PS00477 for a short review).

30       It is believed that the protein of SEQ ID NO: 110 or part thereof is a member of the alpha-2-macroglobulin family, more preferably a protease inhibitor. Preferred polypeptides of the invention are polypeptides comprising the amino acids of SEQ ID NO:110 from positions 17 to 40. Other preferred polypeptides of the invention are fragments of SEQ ID NO:93 having any of the biological activity described herein. The protease inhibitor activity of the protein of the invention or part  
35       thereof may be assessed using any techniques known to those skilled in the art.

      The invention relates to compositions and methods using the protein of the invention or part thereof to inhibit proteases, both in vitro or in vivo. Since proteases play an important role in the

regulation of many biological processes in virtually all living organisms as well as a major role in diseases, inhibitors of proteases are useful in a wide variety of applications.

In one embodiment, the protein of the invention or part thereof may be useful to quantify the amount of a given protease in a biological sample, and thus used in assays and diagnostic kits for the  
5 quantification of proteases in bodily fluids or other tissue samples, in addition to bacterial, fungal, plant, yeast, viral or mammalian cell cultures. In a preferred embodiment, the sample is assayed using a standard protease substrate. A known concentration of protease inhibitor is added, and allowed to bind to a particular protease present. The protease assay is then rerun, and the loss of activity is correlated to the protease inhibitor activity using techniques well known to those skilled in  
10 the art.

In addition, the protein of the invention or part thereof may be used to remove, identify or inhibit contaminating proteases in a sample. Compositions comprising the polypeptides of the present invention may be added to biological samples as a "cocktail" with other protease inhibitors to prevent degradation of protein samples. The advantage of using a cocktail of protease inhibitors  
15 is that one is able to inhibit a wide range of proteases without knowing the specificity of any of the proteases. Using a cocktail of protease inhibitors also protects a protein sample from a wide range of future unknown proteases which may contaminate a protein sample from a vast number of sources. For example, the protein of the invention or part thereof are added to samples where proteolytic degradation by contaminating proteases is undesirable. Such protease inhibitor cocktails  
20 (see for example the ready to use cocktails sold by Sigma) are widely used in research laboratory assays to inhibit proteases susceptible of degrading a protein of interest for which the assay is to be performed. Alternatively, the protein of the invention or part thereof may be bound to a chromatographic support, either alone or in combination with other protease inhibitor, using techniques well known in the art, to form an affinity chromatography column. A sample containing  
25 the undesirable protease is run through the column to remove the protease. Alternatively, the same methods may be used to identify new proteases.

In a preferred embodiment, the protein of the invention or part thereof may be used to inhibit proteases implicated in a number of diseases where cellular proteolysis occur such as diseases characterized by tissue degradation including but not limited to arthritis, muscular dystrophy,  
30 inflammation, tumor invasion, glomerulonephritis, parasite-borne infections, Alzheimer's disease, periodontal disease, and cancer metastasis.

In another preferred embodiment, the protein of the invention or part thereof may be useful to inhibit exogenous proteases, both in vivo and in vitro, implicated in a number of infectious diseases including but not limited to gingivitis, malaria, leishmaniasis, filariasis, osteoporosis and  
35 osteoarthritis, and other bacterial, and parasite-borne or viral infections. In particular, the protein of the invention or part thereof may offer applications in viral diseases where the proteolysis of primary polypeptide precursors is essential to the replication of the virus, as for HIV and HCV.

Furthermore, the protease inhibitors of the present invention find use in drug potentiation applications. For example, therapeutic agents such as antibiotics or antitumor drugs can be inactivated through proteolysis by endogenous proteases, thus rendering the administered drug less effective or inactive. Accordingly, the protease inhibitors of the invention may be administered to a  
5 patient in conjunction with a therapeutic agent in order to potentiate or increase the activity of the drug. This co-administration may be by simultaneous administration, such as a mixture of the protease inhibitor and the drug, or by separate simultaneous or sequential administration.

In addition, protease inhibitors have been shown to inhibit the growth of microorganisms including human pathogenic bacteria. For example, protease inhibitors are able to inhibit growth of all  
10 strains of group A streptococci, including antibiotic-resistant strains (Merigan, T. et al (1996) Ann Intern Med 124:1039-1050; Stoka, V. (1995) FEBS. Lett 370:101-104; Vonderfecht, S. et al (1988) J Clin Invest 82:2011-2016; Collins, A. et al (1991) Antimicrob Agents Chemother 35:2444-2446). Accordingly, the protease inhibitors of the present invention may be used as antibacterial agents to retard or inhibit the growth of certain bacteria either in vitro or in vivo. Particularly, the polypeptides of  
15 the present invention may be used to inhibit the growth of group A streptococci on non-living matter such as instruments not conducive to other methods of preventing or removing contamination by group A streptococci, and in culture of living plant, fungi, and animal cells.

The nucleic acid sequences of SEQ ID NOs: 24-73 or fragments thereof may also be used to construct fusion proteins in which the polypeptide sequences of SEQ ID NOs: 74-123 or fragments  
20 thereof are fused to heterologous polypeptides. For example, the fragments of the polypeptides of SEQ ID NOs. 74-123 which are included in the fusion proteins may comprise at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of the polypeptides of SEQ ID NOs. 74-123 or may be of any length suitable for the intended purpose of the fusion protein. Nucleic acids encoding the desired fusion protein are produced by cloning a nucleic acid of SEQ ID NOs. 24-73 in frame with a  
25 nucleic acid encoding the heterologous polypeptide. The nucleic acid encoding the desired fusion protein is operably linked to a promoter in an appropriate vector, such as any of the vectors described above, and introduced into a host capable of expressing the fusion protein.

Antibodies against the polypeptides of SEQ ID NOs. 74-123 or fragments thereof may be used in immunoaffinity chromatography to isolate the polypeptides of SEQ ID NOs. 74-123 or  
30 fragments thereof or to isolate fusion proteins containing the polypeptides of SEQ ID NOs. 74-123 or fragments thereof.

The invention further relates to methods and compositions using the protein of the invention or part thereof to diagnose, prevent and/or treat several disorders in which the activity of the protein of the invention is deleterious. For diagnostic purposes, the expression of the protein of the  
35 invention could be investigated using any of the Northern blotting, RT-PCR or immunoblotting methods described herein and compared to the expression in control individuals. For prevention and/or treatment purposes, inhibiting the endogenous expression of the protein of the invention using



any of the antisense or triple helix methods described herein may be used. Alternatively, inhibitors for the protein's activity may be developed and use to inhibit and/or reduce its activity using any methods known to those skilled in the art.

Chromosomal localization of the cDNA of the present invention were also determined using  
5 information from public and proprietary databases. Table XI lists the putative chromosomal location of the polynucleotides of the present invention. Column 1 lists the sequence identification number with the corresponding chromosomal location listed in column two.

The present invention also relates to methods and compositions using the chromosomal location of the polynucleotides of the invention to construct a human high resolution map or to identify a given  
10 chromosome in a sample using any techniques to those skilled in the art including those disclosed in Example 43.

Alternatively, the cDNA clone obtained by the process described in Examples 1 through 13 may not include the entire coding sequence of the protein encoded by the corresponding mRNA, although they do include sequences derived from the 5'ends of their corresponding mRNA. Such  
15 5'EST can be used to isolate extended cDNAs which contain sequences adjacent to the 5' ESTs. Such obtained extended cDNAs may include the entire coding sequence of the protein encoded by the corresponding mRNA, including the authentic translation start site. Examples 16 and 17 below describe methods for obtaining extended cDNAs using 5' ESTs. Example 17 also describes methods to obtain cDNA, mRNA or genomic DNA homologous to cDNA, 5'ESTs, or fragment thereof.

20 The methods of Examples 16 and 17 can also be used to obtain cDNAs which encode less than the entire coding sequence of proteins encoded by the genes corresponding to the 5' ESTs. In some embodiments, the cDNAs isolated using these methods encode at least 5, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 consecutive amino acids of one of the proteins encoded by the sequences of SEQ ID NOs. 24-73.

25

#### EXAMPLE 16

##### General Method for Using 5' ESTs to Clone and Sequence cDNAs which Include the Entire Coding Region and the Authentic 5'End of the Corresponding mRNA

The following general method may be used to quickly and efficiently isolate cDNAs including sequence adjacent to the sequences of the 5' ESTs used to obtain them. This method,  
30 illustrated in Figure 3, may be applied to obtain cDNAs for any 5' EST.

The method takes advantage of the known 5' sequence of the mRNA. A reverse transcription reaction is conducted on purified mRNA with a poly dT primer containing a nucleotide sequence at its 5' end allowing the addition of a known sequence at the end of the cDNA which corresponds to the 3' end of the mRNA. Such a primer and a commercially-available reverse  
35 transcriptase enzyme are added to a buffered mRNA sample yielding a reverse transcript anchored at the 3' polyA site of the RNAs. Nucleotide monomers are then added to complete the first strand synthesis. After removal of the mRNA hybridized to the first cDNA strand by alkaline hydrolysis,

the products of the alkaline hydrolysis and the residual poly dT primer can be eliminated with an exclusion column.

Subsequently, a pair of nested primers on each end is designed based on the known 5' sequence from the 5' EST and the known 3' end added by the poly dT primer used in the first strand synthesis. Software used to design primers is either based on GC content and melting temperatures of oligonucleotides, such as OSP (Illier and Green, *PCR Meth. Appl.* 1:124-128, 1991), or based on the octamer frequency disparity method (Griffais *et al.*, *Nucleic Acids Res.* 19: 3887-3891, 1991) such as PC-Rare ([http:// bioinformatics.weizmann.ac.il/software/PC-Rare/doc/manuel.html](http://bioinformatics.weizmann.ac.il/software/PC-Rare/doc/manuel.html)). Preferably, the nested primers at the 5' end and the nested primers at the 3' end are separated from one another by four to nine bases. These primer sequences may be selected to have melting temperatures and specificities suitable for use in PCR.

A first PCR run is performed using the outer primer from each of the nested pairs. A second PCR run using the inner primer from each of the nested pairs is then performed on a small aliquot of the first PCR product. Thereafter, the primers and remaining nucleotide monomers are removed.

Due to the lack of position constraints on the design of 5' nested primers compatible for PCR use using the OSP software, amplicons of two types are obtained. Preferably, the second 5' primer is located upstream of the translation initiation codon thus yielding a nested PCR product containing the entire coding sequence. Such a cDNA may be used in a direct cloning procedure such as the one described in example 4.

However, in some cases, the second 5' primer is located downstream of the translation initiation codon, thereby yielding a PCR product containing only part of the ORF. For such amplicons which do not contain the complete coding sequence, intermediate steps are necessary to obtain both the complete coding sequence and a PCR product containing the full coding sequence. The complete coding sequence can be assembled from several partial sequences determined directly from different PCR products. Once the full coding sequence has been completely determined, new primers compatible for PCR use are then designed to obtain amplicons containing the whole coding region. However, in such cases, 3' primers compatible for PCR use are located inside the 3' UTR of the corresponding mRNA, thus yielding amplicons which lack part of this region, *i.e.* the polyA tract and sometimes the polyadenylation signal, as illustrated in Figure 3. Such obtained cDNAs are then cloned into an appropriate vector using a procedure essentially similar to the one described in example 4.

Full-length PCR products are then sequenced using a procedure similar to the one described in example 11. Completion of the sequencing of a given cDNA fragment may be assessed by comparing the sequence length to the size of the corresponding nested PCR product. When Northern blot data are available, the size of the mRNA detected for a given PCR product may also be used to finally assess that the sequence is complete. Sequences which do not fulfill these criteria are discarded and will undergo a new isolation procedure.

Full-length PCR products are then cloned in an appropriate vector. For example, the cDNAs can be cloned into a vector using a procedure similar to the one described in example 4. Such full-length cDNA clones are then double-sequenced and submitted to computer analyses using procedure essentially similar to the ones described in Examples 11 through 13. However, it will be appreciated that full-length cDNA clones obtained from amplicons lacking part of the 3'UTR may lack polyadenylations sites and signals.

#### EXAMPLE 17

##### Methods for Obtaining cDNAs or Nucleic Acids Homologous to cDNAs or Fragments Thereof

In addition to PCR based methods for obtaining cDNAs, traditional hybridization based methods may also be employed. These methods may also be used to obtain the genomic DNAs which encode the mRNAs from which the cDNA is derived, mRNAs corresponding to the cDNAs, or nucleic acids which are homologous to cDNAs or fragments thereof. Indeed, cDNAs of the present invention or fragments thereof, including 5'ESTs, may also be used to isolate cDNAs or nucleic acids homologous to cDNAs from a cDNA library or a genomic DNA library as follows. Such cDNA libraries or genomic DNA libraries may be obtained from a commercial source or made using techniques familiar to those skilled in the art such as the one described in Examples 1 through 5. An example of such hybridization-based methods is provided below. Techniques for identifying cDNA clones in a cDNA library which hybridize to a given probe sequence are disclosed in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual 2d Ed.*, Cold Spring Harbor Laboratory Press, 1989, the disclosure of which is incorporated herein by reference. The same techniques may be used to isolate genomic DNAs.

Briefly, cDNA or genomic DNA clones which hybridize to the detectable probe are identified and isolated for further manipulation as follows. A probe comprising at least 10 consecutive nucleotides from the cDNA or fragment thereof is labeled with a detectable label such as a radioisotope or a fluorescent molecule. Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the cDNA or fragment thereof. More preferably, the probe comprises 20 to 30 consecutive nucleotides from the cDNA or fragment thereof. In some embodiments, the probe comprises more than 30 nucleotides from the cDNA or fragment thereof.

Techniques for labeling the probe are well known and include phosphorylation with polynucleotide kinase, nick translation, *in vitro* transcription, and non radioactive techniques. The cDNAs or genomic DNAs in the library are transferred to a nitrocellulose or nylon filter and denatured. After blocking of non specific sites, the filter is incubated with the labeled probe for an amount of time sufficient to allow binding of the probe to cDNAs or genomic DNAs containing a sequence capable of hybridizing thereto.

By varying the stringency of the hybridization conditions used to identify cDNAs or genomic DNAs which hybridize to the detectable probe, cDNAs or genomic DNAs having different levels of identity to the probe can be identified and isolated as described below.

### 1. Isolation of cDNA or Genomic DNA Sequences Having a High Degree of Identity to the Labeled Probe

To identify cDNAs or genomic DNAs having a high degree of identity to the probe sequence, the melting temperature of the probe may be calculated using the following formulas:

- 5 For probes between 14 and 70 nucleotides in length the melting temperature ( $T_m$ ) is calculated using the formula:  $T_m = 81.5 + 16.6(\log(Na^+)) + 0.41(\text{fraction } G+C) - (600/N)$  where  $N$  is the length of the probe.

- If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation  $T_m = 81.5 + 16.6(\log(Na^+)) + 0.41(\text{fraction } G+C) - 10(0.63\% \text{ formamide}) - (600/N)$  where  $N$  is the length of the probe.

Prehybridization may be carried out in 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100  $\mu$ g denatured fragmented salmon sperm DNA or 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100  $\mu$ g denatured fragmented salmon sperm DNA, 50% formamide. The formulas for SSC and Denhardt's solutions are listed in Sambrook *et al.*, *supra*.

- 15 Hybridization is conducted by adding the detectable probe to the prehybridization solutions listed above. Where the probe comprises double stranded DNA, it is denatured before addition to the hybridization solution. The filter is contacted with the hybridization solution for a sufficient period of time to allow the probe to hybridize to cDNAs or genomic DNAs containing sequences complementary thereto or homologous thereto. For probes over 200 nucleotides in length, the 20 hybridization may be carried out at 15-25°C below the  $T_m$ . For shorter probes, such as oligonucleotide probes, the hybridization may be conducted at 15-25°C below the  $T_m$ . Preferably, for hybridizations in 6X SSC, the hybridization is conducted at approximately 68°C. Preferably, for hybridizations in 50% formamide containing solutions, the hybridization is conducted at approximately 42°C.

- 25 All of the foregoing hybridizations would be considered to be under "stringent" conditions. Following hybridization, the filter is washed in 2X SSC, 0.1% SDS at room temperature for 15 minutes. The filter is then washed with 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour. Thereafter, the solution is washed at the hybridization temperature in 0.1X SSC, 0.5% SDS. A final wash is conducted in 0.1X SSC at room temperature.

- 30 cDNAs or genomic DNAs which have hybridized to the probe are identified by autoradiography or other conventional techniques.

### 2. Isolation of cDNA or Genomic DNA Sequences Having Lower Degrees of Identity to the Labeled Probe

- The above procedure may be modified to identify cDNAs or genomic DNAs having 35 decreasing levels of identity to the probe sequence. For example, to obtain cDNAs or genomic DNAs of decreasing identity to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C

in a hybridization buffer having a sodium concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C.

- 5 Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of identity to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 10 25% formamide and "low" conditions below 25% formamide. cDNAs or genomic DNAs which have hybridized to the probe are identified by autoradiography or other conventional techniques.

3. Determination of the Degree of Identity between the Obtained cDNAs or Genomic DNAs and cDNAs or Fragments thereof Used as the Labeled Probe or Between the Polypeptides Encoded by the Obtained cDNAs or Genomic DNAs and the Polypeptides Encoded by the cDNAs or Fragment  
15 Thereof Used as the Labeled Probe

- To determine the level of identity between the hybridized cDNA or genomic DNA and the cDNA or fragment thereof from which the probe was derived, the nucleotide sequences of the hybridized nucleic acid and the cDNA or fragment thereof from which the probe was derived are compared. The sequences of the cDNA or fragment thereof from which the probe was derived and 20 the sequences of the cDNA or genomic DNA which hybridized to the detectable probe may be stored on a computer readable medium as described below and compared to one another using any of a variety of algorithms familiar to those skilled in the art such as those described below.

- To determine the level of identity between the polypeptide encoded by the hybridizing cDNA or genomic DNA and the polypeptide encoded by the cDNA or fragment thereof from which 25 the probe was derived, the polypeptide sequence encoded by the hybridized nucleic acid and the polypeptide sequence encoded by the cDNA or fragment thereof from which the probe was derived are compared. The sequences of the polypeptide encoded by the cDNA or fragment thereof from which the probe was derived and the polypeptide sequence encoded by the cDNA or genomic DNA which hybridized to the detectable probe may be stored on a computer readable medium as described 30 below and compared to one another using any of a variety of algorithms familiar to those skilled in the art such as those described below.

- Protein and/or nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW 35 (Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85(8):2444-2448; Altschul *et al.*, 1990, *J. Mol. Biol.* 215(3):403-410; Thompson *et al.*, 1994, *Nucleic Acids Res.* 22(2):4673-4680; Higgins *et*

*al.*, 1996, *Methods Enzymol.* 266:383-402; Altschul *et al.*, 1990, *J. Mol. Biol.* 215(3):403-410; Altschul *et al.*, 1993, *Nature Genetics* 3:266-272).

In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") which is well known in the art (see, *e.g.*, Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2267-2268; Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-410; Altschul *et al.*, 1993, *Nature Genetics* 3:266-272; Altschul *et al.*, 1997, *Nuc. Acids Res.* 25:3389-3402). In particular, five specific BLAST programs are used to perform the following task:

- (1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;
- (2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;
- (3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;
- (4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and
- (5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (*i.e.*, aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet *et al.*, 1992, *Science* 256:1443-1445; Henikoff and Henikoff, 1993, *Proteins* 17:49-61). Less preferably, the PAM or PAM250 matrices may also be used (see, *e.g.*, Schwartz and Dayhoff, eds., 1978, *Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure*, Washington: National Biomedical Research Foundation)

The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent identity. Preferably, the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (see, *e.g.*, Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2267-2268).

The parameters used with the above algorithms may be adapted depending on the sequence length and degree of identity studied. In some embodiments, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user.

In some embodiments, the level of identity between the hybridized nucleic acid and the cDNA or fragment thereof from which the probe was derived may be determined using the FASTDB algorithm described in Brutlag *et al.* *Comp. App. Biosci.* 6:237-245, 1990. In such analyses the

parameters may be selected as follows: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the sequence which hybridizes to the probe, whichever is shorter. Because the FASTDB program does not consider 5' or 3' truncations when

5 calculating identity levels, if the sequence which hybridizes to the probe is truncated relative to the sequence of the cDNA or fragment thereof from which the probe was derived the identity level is manually adjusted by calculating the number of nucleotides of the cDNA or fragment thereof which are not matched or aligned with the hybridizing sequence, determining the percentage of total nucleotides of the hybridizing sequence which the non-matched or non-aligned nucleotides

10 represent, and subtracting this percentage from the identity level. For example, if the hybridizing sequence is 700 nucleotides in length and the cDNA or fragment thereof sequence is 1000 nucleotides in length wherein the first 300 bases at the 5' end of the cDNA or fragment thereof are absent from the hybridizing sequence, and wherein the overlapping 700 nucleotides are identical, the identity level would be adjusted as follows. The non-matched, non-aligned 300 bases represent 30%

15 of the length of the cDNA or fragment thereof. If the overlapping 700 nucleotides are 100% identical, the adjusted identity level would be  $100-30=70\%$  identity. It should be noted that the preceding adjustments are only made when the non-matched or non-aligned nucleotides are at the 5' or 3' ends. No adjustments are made if the non-matched or non-aligned sequences are internal or under any other conditions.

20 For example, using the above methods, nucleic acids having at least 95% nucleic acid identity, at least 96% nucleic acid identity, at least 97% nucleic acid identity, at least 98% nucleic acid identity, at least 99% nucleic acid identity, or more than 99% nucleic acid identity to the cDNA or fragment thereof from which the probe was derived may be obtained and identified. Such nucleic acids may be allelic variants or related nucleic acids from other species. Similarly, by using

25 progressively less stringent hybridization conditions one can obtain and identify nucleic acids having at least 90%, at least 85%, at least 80% or at least 75% identity to the cDNA or fragment thereof from which the probe was derived.

Using the above methods and algorithms such as FASTA with parameters depending on the sequence length and degree of identity studied, for example the default parameters used by the

30 algorithms in the absence of instructions from the user, one can obtain nucleic acids encoding proteins having at least 99%, at least 98%, at least 97%, at least 96%, at least 95%, at least 90%, at least 85%, at least 80% or at least 75% identity to the protein encoded by the cDNA or fragment thereof from which the probe was derived. In some embodiments, the identity levels can be determined using the "default" opening penalty and the "default" gap penalty, and a scoring matrix

35 such as PAM 250 (a standard scoring matrix; see Dayhoff *et al.*, in: Atlas of Protein Sequence and Structure, Vol. 5, Supp. 3 (1978)).

Alternatively, the level of polypeptide identity may be determined using the FASTDB algorithm described by Brutlag *et al.* Comp. App. Biosci. 6:237-245, 1990. In such analyses the parameters may be selected as follows: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=Sequence Length, Gap  
5 Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the homologous sequence, whichever is shorter. If the homologous amino acid sequence is shorter than the amino acid sequence encoded by the cDNA or fragment thereof as a result of an N terminal and/or C terminal deletion the results may be manually corrected as follows. First, the number of amino acid residues of the amino acid sequence encoded by the cDNA or fragment thereof which are not matched or  
10 aligned with the homologous sequence is determined. Then, the percentage of the length of the sequence encoded by the cDNA or fragment thereof which the non-matched or non-aligned amino acids represent is calculated. This percentage is subtracted from the identity level. For example wherein the amino acid sequence encoded by the cDNA or fragment thereof is 100 amino acids in length and the length of the homologous sequence is 80 amino acids and wherein the amino acid  
15 sequence encoded by the cDNA or fragment thereof is truncated at the N terminal end with respect to the homologous sequence, the identity level is calculated as follows. In the preceding scenario there are 20 non-matched, non-aligned amino acids in the sequence encoded by the cDNA or fragment thereof. This represents 20% of the length of the amino acid sequence encoded by the cDNA or fragment thereof. If the remaining amino acids are 100% identical between the two  
20 sequences, the identity level would be  $100\% - 20\% = 80\%$  identity. No adjustments are made if the non-matched or non-aligned sequences are internal or under any other conditions.

In addition to the above described methods, other protocols are available to obtain homologous cDNAs using cDNA of the present invention or fragment thereof as outlined in the following paragraphs.

25 cDNAs may be prepared by obtaining mRNA from the tissue, cell, or organism of interest using mRNA preparation procedures utilizing polyA selection procedures or other techniques known to those skilled in the art. A first primer capable of hybridizing to the polyA tail of the mRNA is hybridized to the mRNA and a reverse transcription reaction is performed to generate a first cDNA strand.

30 The first cDNA strand is hybridized to a second primer containing at least 10 consecutive nucleotides of the sequences of SEQ ID NOs 24-73. Preferably, the primer comprises at least 10, 12, 15, 17, 18, 20, 23, 25, or 28 consecutive nucleotides from the sequences of SEQ ID NOs 24-73. In some embodiments, the primer comprises more than 30 nucleotides from the sequences of SEQ ID NOs 24-73. If it is desired to obtain cDNAs containing the full protein coding sequence,  
35 including the authentic translation initiation site, the second primer used contains sequences located upstream of the translation initiation site. The second primer is extended to generate a second cDNA



strand complementary to the first cDNA strand. Alternatively, RT-PCR may be performed as described above using primers from both ends of the cDNA to be obtained.

cDNAs containing 5' fragments of the mRNA may be prepared by hybridizing an mRNA comprising the sequences of SEQ ID NOs. 24-73 with a primer comprising a complementary to a  
5 fragment of the known cDNA, genomic DNA or fragment thereof hybridizing the primer to the mRNAs, and reverse transcribing the hybridized primer to make a first cDNA strand from the mRNAs. Preferably, the primer comprises at least 10, 12, 15, 17, 18, 20, 23, 25, or 28 consecutive nucleotides of the sequences complementary to SEQ ID NOs. 24-73.

Thereafter, a second cDNA strand complementary to the first cDNA strand is synthesized.  
10 The second cDNA strand may be made by hybridizing a primer complementary to sequences in the first cDNA strand to the first cDNA strand and extending the primer to generate the second cDNA strand.

The double stranded cDNAs made using the methods described above are isolated and cloned. The cDNAs may be cloned into vectors such as plasmids or viral vectors capable of  
15 replicating in an appropriate host cell. For example, the host cell may be a bacterial, mammalian, avian, or insect cell.

Techniques for isolating mRNA, reverse transcribing a primer hybridized to mRNA to generate a first cDNA strand, extending a primer to make a second cDNA strand complementary to the first cDNA strand, isolating the double stranded cDNA and cloning the double stranded cDNA  
20 are well known to those skilled in the art and are described in *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. 1997 and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989.

Alternatively, other procedures may be used for obtaining full-length cDNAs or homologous cDNAs. In one approach, cDNAs are prepared from mRNA and cloned into double stranded  
25 phagemids as follows. The cDNA library in the double stranded phagemids is then rendered single stranded by treatment with an endonuclease, such as the Gene II product of the phage F1 and an exonuclease (Chang *et al.*, *Gene* 127:95-8, 1993). A biotinylated oligonucleotide comprising the sequence of a fragment of a known cDNA, genomic DNA or fragment thereof is hybridized to the single stranded phagemids. Preferably, the fragment comprises at least 10, 12, 15, 17, 18, 20, 23, 25,  
30 or 28 consecutive nucleotides of the sequences of SEQ ID NOs. 24-73.

Hybrids between the biotinylated oligonucleotide and phagemids are isolated by incubating the hybrids with streptavidin coated paramagnetic beads and retrieving the beads with a magnet (Fry *et al.*, *Biotechniques*, 13: 124-131, 1992). Thereafter, the resulting phagemids are released from the beads and converted into double stranded DNA using a primer specific for the cDNA or fragment  
35 thereof used to design the biotinylated oligonucleotide. Alternatively, protocols such as the Gene Trapper kit (Gibco BRL) may be used. The resulting double stranded DNA is transformed into

bacteria. Homologous cDNAs or full length cDNAs containing the cDNA or fragment thereof sequence are identified by colony PCR or colony hybridization.

Using any of the above described methods, a plurality of cDNAs containing full-length protein coding sequences or fragments of the protein coding sequences may be provided as cDNA  
5 libraries for subsequent evaluation of the encoded proteins or use in diagnostic assays as described below.

cDNAs prepared by any method described therein may be subsequently engineered to obtain nucleic acids which include desired fragments of the cDNA using conventional techniques such as subcloning, PCR, or *in vitro* oligonucleotide synthesis. For example, nucleic acids which include  
10 only the full coding sequences (*i.e.* the sequences encoding the signal peptide and the mature protein remaining after the signal peptide peptide is cleaved off) may be obtained using techniques known to those skilled in the art. Alternatively, conventional techniques may be applied to obtain nucleic acids which contain only the coding sequence for the mature protein remaining after the signal peptide is cleaved off or nucleic acids which contain only the coding sequences for the signal  
15 peptides.

Similarly, nucleic acids containing any other desired fragment of the coding sequences for the encoded protein may be obtained. For example, the nucleic acid may contain at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 consecutive bases of a cDNA.

20 Once a cDNA has been obtained, it can be sequenced to determine the amino acid sequence it encodes. Once the encoded amino acid sequence has been determined, one can create and identify any of the many conceivable cDNAs that will encode that protein by simply using the degeneracy of the genetic code. For example, allelic variants or other homologous nucleic acids can be identified as described below. Alternatively, nucleic acids encoding the desired amino acid sequence can be  
25 synthesized *in vitro*.

In a preferred embodiment, the coding sequence may be selected using the known codon or codon pair preferences for the host organism in which the cDNA is to be expressed.

#### IV. Use of cDNA or Fragments Thereof to Express Proteins and Uses of Those Expressed Proteins

Using any of the above described methods, cDNAs containing the full protein coding  
30 sequences of their corresponding mRNAs or portions thereof, such as cDNAs encoding the mature protein, may be used to express the secreted proteins or portions thereof which they encode as described below. If desired, the cDNAs may contain the sequences encoding the signal peptide to facilitate secretion of the expressed protein. It will be appreciated that a plurality of extended cDNAs containing the full protein coding sequences or portions thereof may be simultaneously  
35 cloned into expression vectors to create an expression library for analysis of the encoded proteins as described below.

#### **EXAMPLE 18**

Expression of the Proteins Encoded by cDNAs or Fragments Thereof

To express the proteins encoded by the cDNAs or fragments thereof, nucleic acids containing the coding sequence for the proteins or fragments thereof to be expressed are obtained as described above and cloned into a suitable expression vector. If desired, the nucleic acids may  
5 contain the sequences encoding the signal peptide to facilitate secretion of the expressed protein. For example, the nucleic acid may comprise the sequence of one of SEQ ID NOs: 24-73 listed in Table I and in the accompanying sequence listing. Alternatively, the nucleic acid may comprise those nucleotides which make up the full coding sequence of one of the sequences of SEQ ID NOs: 24-73 as defined in Table I above.

10 It will be appreciated that should the extent of the full coding sequence (i.e. the sequence encoding the signal peptide and the mature protein resulting from cleavage of the signal peptide) differ from that listed in Table I as a result of a sequencing error, reverse transcription or amplification error, mRNA splicing, post-translational modification of the encoded protein, enzymatic cleavage of the encoded protein, or other biological factors, one skilled in the art would  
15 be readily able to identify the extent of the full coding sequences in the sequences of SEQ ID NOs. 24-73. Accordingly, the scope of any claims herein relating to nucleic acids containing the full coding sequence of one of SEQ ID NOs. 24-73 is not to be construed as excluding any readily identifiable variations from or equivalents to the full coding sequences listed in Table I. Similarly, should the extent of the full length polypeptides differ from those indicated in Table II as a result of  
20 any of the preceding factors, the scope of claims relating to polypeptides comprising the amino acid sequence of the full length polypeptides is not to be construed as excluding any readily identifiable variations from or equivalents to the sequences listed in Table II.

Alternatively, the nucleic acid used to express the protein or fragment thereof may comprise those nucleotides which encode the mature protein (i.e. the protein created by cleaving the signal  
25 peptide off) encoded by one of the sequences of SEQ ID NOs: 24-73 as defined in Table I above.

It will be appreciated that should the extent of the sequence encoding the mature protein differ from that listed in Table I as a result of a sequencing error, reverse transcription or amplification error, mRNA splicing, post-translational modification of the encoded protein, enzymatic cleavage of the encoded protein, or other biological factors, one skilled in the art would  
30 be readily able to identify the extent of the sequence encoding the mature protein in the sequences of SEQ ID NOs. 24-73. Accordingly, the scope of any claims herein relating to nucleic acids containing the sequence encoding the mature protein encoded by one of SEQ ID NOs. 24-73 is not to be construed as excluding any readily identifiable variations from or equivalents to the sequences listed in Table I. Thus, claims relating to nucleic acids containing the sequence encoding the mature  
35 protein encompass equivalents to the sequences listed in Table I, such as sequences encoding biologically active proteins resulting from post-translational modification, enzymatic cleavage, or other readily identifiable variations from or equivalents to the secreted proteins in addition to

cleavage of the signal peptide. Similarly, should the extent of the mature polypeptides differ from those indicated in Table II as a result of any of the preceding factors, the scope of claims relating to polypeptides comprising the sequence of a mature protein included in the sequence of one of SEQ ID NOs. 74-123 is not to be construed as excluding any readily identifiable variations from or  
5 equivalents to the sequences listed in Table II. Thus, claims relating to polypeptides comprising the sequence of the mature protein encompass equivalents to the sequences listed in Table II, such as biologically active proteins resulting from post-translational modification, enzymatic cleavage, or other readily identifiable variations from or equivalents to the secreted proteins in addition to cleavage of the signal peptide. It will also be appreciated that should the biologically active form of  
10 the polypeptides included in the sequence of one of SEQ ID NOs. 74-123 or the nucleic acids encoding the biologically active form of the polypeptides differ from those identified as the mature polypeptide in Table II or the nucleotides encoding the mature polypeptide in Table I as a result of a sequencing error, reverse transcription or amplification error, mRNA splicing, post-translational modification of the encoded protein, enzymatic cleavage of the encoded protein, or other biological  
15 factors, one skilled in the art would be readily able to identify the amino acids in the biologically active form of the polypeptides and the nucleic acids encoding the biologically active form of the polypeptides. In such instances, the claims relating to polypeptides comprising the mature protein included in one of SEQ ID NOs. 74-123 or nucleic acids comprising the nucleotides of one of SEQ ID NOs. 24-73 encoding the mature protein shall not be construed to exclude any readily identifiable  
20 variations from the sequences listed in Table I and Table II.

In some embodiments, the nucleic acid used to express the protein or fragment thereof may comprise those nucleotides which encode the signal peptide encoded by one of the sequences of SEQ ID NOs: 24-73 as defined in Table I above.

It will be appreciated that should the extent of the sequence encoding the signal peptide  
25 differ from that listed in Table I as a result of a sequencing error, reverse transcription or amplification error, mRNA splicing, post-translational modification of the encoded protein, enzymatic cleavage of the encoded protein, or other biological factors, one skilled in the art would be readily able to identify the extent of the sequence encoding the signal peptide in the sequences of SEQ ID NOs. 24-73. Accordingly, the scope of any claims herein relating to nucleic acids  
30 containing the sequence encoding the signal peptide encoded by one of SEQ ID NOs. 24-73 is not to be construed as excluding any readily identifiable variations from the sequences listed in Table I. Similarly, should the extent of the signal peptides differ from those indicated in Table II as a result of any of the preceding factors, the scope of claims relating to polypeptides comprising the sequence of a signal peptide included in the sequence of one of SEQ ID NOs. 74-123 is not to be construed as  
35 excluding any readily identifiable variations from the sequences listed in Table II.

Alternatively, the nucleic acid may encode a polypeptide comprising at least 5 consecutive amino acids of one of the sequences of SEQ ID NOs: 74-123. In some embodiments, the nucleic

acid may encode a polypeptide comprising at least 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 consecutive amino acids of one of the sequences of SEQ ID NOs: 74-123.

The nucleic acids inserted into the expression vectors may also contain sequences upstream of the sequences encoding the signal peptide, such as sequences which regulate expression levels or  
5 sequences which confer tissue specific expression.

The nucleic acid encoding the protein or polypeptide to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology. The expression vector may be any of the mammalian, yeast, insect or bacterial expression systems known in the art. Commercially available vectors and expression systems are available from a variety of suppliers  
10 including Genetics Institute (Cambridge, MA), Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism in which the expression vector is introduced, as explained by Hatfield, *et al.*, U.S. Patent No. 5,082,767, incorporated herein by this reference.

15 The following is provided as one exemplary method to express the proteins encoded by the cDNAs or the nucleic acids described above. First, the methionine initiation codon for the gene and the poly A signal of the gene are identified. If the nucleic acid encoding the polypeptide to be expressed lacks a methionine to serve as the initiation site, an initiating methionine can be introduced next to the first codon of the nucleic acid using conventional techniques. Similarly, if the  
20 cDNA lacks a poly A signal, this sequence can be added to the construct by, for example, splicing out the Poly A signal from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a fragment of the *gag* gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex  
25 Thymidine Kinase promoter and the selectable neomycin gene. The cDNA or fragment thereof encoding the polypeptide to be expressed is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the cDNA or fragment thereof and containing restriction endonuclease sequences for Pst I incorporated into the 5' primer and BglII at the 5' end of the corresponding cDNA 3' primer, taking care to ensure that the cDNA is positioned in frame with the  
30 poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and digested with BglII.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification.  
35 Positive transfectants are selected after growing the transfected cells in 600ug/ml G418 (Sigma, St. Louis, Missouri). Preferably the expressed protein is released into the culture medium, thereby facilitating purification.

Alternatively, the cDNAs may be cloned into pED6dpc2 (DiscoverEase, Genetics Institute, Cambridge, MA). The resulting pED6dpc2 constructs may be transfected into a suitable host cell, such as COS 1 cells. Methotrexate resistant cells are selected and expanded. Preferably, the protein expressed from the cDNA is released into the culture medium thereby facilitating purification.

- 5 Proteins in the culture medium are separated by gel electrophoresis. If desired, the proteins may be ammonium sulfate precipitated or separated based on size or charge prior to electrophoresis.

As a control, the expression vector lacking a cDNA insert is introduced into host cells or organisms and the proteins in the medium are harvested. The secreted proteins present in the medium are detected using techniques such as Coomassie or silver staining or using antibodies  
10 against the protein encoded by the cDNA. Coomassie and silver staining techniques are familiar to those skilled in the art.

Antibodies capable of specifically recognizing the protein of interest may be generated using synthetic 15-mer peptides having a sequence encoded by the appropriate 5' EST, cDNA, or fragment thereof. The synthetic peptides are injected into mice to generate antibody to the polypeptide  
15 encoded by the 5' EST, cDNA, or fragment thereof.

Secreted proteins from the host cells or organisms containing an expression vector which contains the cDNA or a fragment thereof are compared to those from the control cells or organism. The presence of a band in the medium from the cells containing the expression vector which is absent in the medium from the control cells indicates that the cDNA encodes a secreted protein.  
20 Generally, the band corresponding to the protein encoded by the cDNA will have a mobility near that expected based on the number of amino acids in the open reading frame of the cDNA. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

Alternatively, if the protein expressed from the above expression vectors does not contain  
25 sequences directing its secretion, the proteins expressed from host cells containing an expression vector containing an insert encoding a secreted protein or fragment thereof can be compared to the proteins expressed in host cells containing the expression vector without an insert. The presence of a band in samples from cells containing the expression vector with an insert which is absent in samples from cells containing the expression vector without an insert indicates that the desired  
30 protein or fragment thereof is being expressed. Generally, the band will have the mobility expected for the secreted protein or fragment thereof. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

The protein encoded by the cDNA may be purified using standard immunochromatography  
35 techniques. In such procedures, a solution containing the secreted protein, such as the culture medium or a cell extract, is applied to a column having antibodies against the secreted protein attached to the chromatography matrix. The secreted protein is allowed to bind the

immunochromatography column. Thereafter, the column is washed to remove non-specifically bound proteins. The specifically bound secreted protein is then released from the column and recovered using standard techniques.

If antibody production is not possible, the cDNA sequence or fragment thereof may be incorporated into expression vectors designed for use in purification schemes employing chimeric polypeptides. In such strategies the coding sequence of the cDNA or fragment thereof is inserted in frame with the gene encoding the other half of the chimera. The other half of the chimera may be  $\beta$ -globin or a nickel binding polypeptide encoding sequence. A chromatography matrix having antibody to  $\beta$ -globin or nickel attached thereto is then used to purify the chimeric protein. Protease cleavage sites may be engineered between the  $\beta$ -globin gene or the nickel binding polypeptide and the cDNA or fragment thereof. Thus, the two polypeptides of the chimera may be separated from one another by protease digestion.

One useful expression vector for generating  $\beta$ -globin chimerics is pSG5 (Stratagene), which encodes rabbit  $\beta$ -globin. Intron II of the rabbit  $\beta$ -globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis *et al.*, (Basic Methods in Molecular Biology, L.G. Davis, M.D. Digner, and J.F. Battey, ed., Elsevier Press, NY, 1986) and many of the methods are available from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from the construct using *in vitro* translation systems such as the *In vitro* Express<sup>TM</sup> Translation Kit (Stratagene).

Following expression and purification of the secreted proteins encoded by the 5' ESTs, cDNAs, or fragments thereof, the purified proteins may be tested for the ability to bind to the surface of various cell types as described below. It will be appreciated that a plurality of proteins expressed from these cDNAs may be included in a panel of proteins to be simultaneously evaluated for the activities specifically described below, as well as other biological roles for which assays for determining activity are available.

Alternatively, the polypeptide to be expressed may also be a product of transgenic animals, i.e., as a component of the milk of transgenic cows, goats, pigs or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein of interest.

#### EXAMPLE 19

##### Analysis of Secreted Proteins to Determine Whether they Bind to the Cell Surface

The proteins encoded by the cDNAs, or fragments thereof are cloned into expression vectors such as those described in the previous example. The proteins are purified by size, charge, immunochromatography or other techniques familiar to those skilled in the art. Following purification, the proteins are labeled using techniques known to those skilled in the art. The labeled proteins are incubated with cells or cell lines derived from a variety of organs or tissues to allow the

proteins to bind to any receptor present on the cell surface. Following the incubation, the cells are washed to remove non-specifically bound protein. The labeled proteins are detected by autoradiography. Alternatively, unlabeled proteins may be incubated with the cells and detected with antibodies having a detectable label, such as a fluorescent molecule, attached thereto.

5        Specificity of cell surface binding may be analyzed by conducting a competition analysis in which various amounts of unlabeled protein are incubated along with the labeled protein. The amount of labeled protein bound to the cell surface decreases as the amount of competitive unlabeled protein increases. As a control, various amounts of an unlabeled protein unrelated to the labeled protein is included in some binding reactions. The amount of labeled protein bound to the cell  
10 surface does not decrease in binding reactions containing increasing amounts of unrelated unlabeled protein, indicating that the protein encoded by the cDNA binds specifically to the cell surface.

As discussed above, secreted proteins have been shown to have a number of important physiological effects and, consequently, represent a valuable therapeutic resource. The secreted proteins encoded by the cDNAs or fragments thereof made using any of the methods described  
15 therein may be evaluated to determine their physiological activities as described below.

#### EXAMPLE 20

##### Assaying the Proteins Expressed from cDNAs or Fragments Thereof for Cytokine, Cell Proliferation or Cell Differentiation Activity

As discussed above, secreted proteins may act as cytokines or may affect cellular  
20 proliferation or differentiation. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11,  
25 BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7c and CMK. The proteins encoded by the above cDNAs or fragments thereof may be evaluated for their ability to regulate T cell or thymocyte proliferation in assays such as those described above or in the following references, which are incorporated herein by reference: Current Protocols in Immunology, Ed. by J.E. Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience; Takai *et al. J. Immunol.*  
30 137:3494-3500, 1986. Bertagnolli *et al. J. Immunol.* 145:1706-1712, 1990. Bertagnolli *et al., Cellular Immunology* 133:327-341, 1991. Bertagnolli, *et al. J. Immunol.* 149:3778-3783, 1992; Bowman *et al., J. Immunol.* 152:1756-1761, 1994.

In addition, numerous assays for cytokine production and/or the proliferation of spleen cells, lymph node cells and thymocytes are known. These include the techniques disclosed in Current  
35 Protocols in Immunology. J.E. Coligan *et al.* Eds., Vol 1 pp. 3.12.1-3.12.14 John Wiley and Sons, Toronto, 1994; and Schreiber, R.D. Current Protocols in Immunology, *supra* Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto, 1994.



The proteins encoded by the cDNAs may also be assayed for the ability to regulate the proliferation and differentiation of hematopoietic or lymphopoietic cells. Many assays for such activity are familiar to those skilled in the art, including the assays in the following references, which are incorporated herein by reference: Bottomly, K., Davis, L.S. and Lipsky, P.E.,  
 5 Measurement of Human and Murine Interleukin 2 and Interleukin 4, Current Protocols in Immunology, J.E. Coligan *et al.* Eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries *et al.*, *J. Exp. Med.* 173:1205-1211, 1991; Moreau *et al.*, *Nature* 36:690-692, 1988; Greenberger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-2938, 1983; Nordan, R., Measurement of Mouse and Human Interleukin 6 Current Protocols in Immunology. J.E. Coligan *et al.* Eds. Vol 1  
 10 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 83:1857-1861, 1986; Bennett, F., Giannotti, J., Clark, S.C. and Turner, K.J., Measurement of Human Interleukin 11 Current Protocols in Immunology. J.E. Coligan *et al.* Eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J., Measurement of Mouse and Human Interleukin 9 Current Protocols in Immunology. J.E. Coligan *et al.*,  
 15 *al.*, Eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

The proteins encoded by the cDNAs may also be assayed for their ability to regulate T-cell responses to antigens. Many assays for such activity are familiar to those skilled in the art, including the assays described in the following references, which are incorporated herein by reference: Chapter 3 (*In vitro* Assays for Mouse Lymphocyte Function), Chapter 6 (Cytokines and Their  
 20 Cellular Receptors) and Chapter 7, (Immunologic Studies in Humans) in Current Protocols in Immunology, J.E. Coligan *et al.* Eds. Greene Publishing Associates and Wiley-Interscience; Weinberger *et al.*, *Proc. Natl. Acad. Sci. USA* 77:6091-6095, 1980; Weinberger *et al.*, *Eur. J. Immun.* 11:405-411, 1981; Takai *et al.*, *J. Immunol.* 137:3494-3500, 1986; Takai *et al.*, *J. Immunol.* 140:508-512, 1988.

25 Those proteins which exhibit cytokine, cell proliferation, or cell differentiation activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which induction of cell proliferation or differentiation is beneficial. Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as  
 30 desired.

## EXAMPLE 21

### Assaying the Proteins Expressed from cDNAs or Fragments

#### Thereof for Activity as Immune System Regulators

The proteins encoded by the cDNAs may also be evaluated for their effects as immune  
 35 regulators. For example, the proteins may be evaluated for their activity to influence thymocyte or splenocyte cytotoxicity. Numerous assays for such activity are familiar to those skilled in the art including the assays described in the following references, which are incorporated herein by

reference: Chapter 3 (*In vitro* Assays for Mouse Lymphocyte Function 3.1-3.19) and Chapter 7 (Immunologic studies in Humans) in Current Protocols in Immunology, J.E. Coligan *et al.* Eds, Greene Publishing Associates and Wiley-Interscience; Herrmann *et al.*, *Proc. Natl. Acad. Sci. USA* 78:2488-2492, 1981; Herrmann *et al.*, *J. Immunol.* 128:1968-1974, 1982; Handa *et al.*, *J. Immunol.* 135:1564-1572, 1985; Takai *et al.*, *J. Immunol.* 137:3494-3500, 1986; Takai *et al.*, *J. Immunol.* 140:508-512, 1988; Herrmann *et al.*, *Proc. Natl. Acad. Sci. USA* 78:2488-2492, 1981; Herrmann *et al.*, *J. Immunol.* 128:1968-1974, 1982; Handa *et al.*, *J. Immunol.* 135:1564-1572, 1985; Takai *et al.*, *J. Immunol.* 137:3494-3500, 1986; Bowman *et al.*, *J. Virology* 61:1992-1998; Takai *et al.*, *J. Immunol.* 140:508-512, 1988; Bertagnolli *et al.*, *Cellular Immunology* 133:327-341, 1991; Brown *et al.*, *J. Immunol.* 153:3079-3092, 1994.

The proteins encoded by the cDNAs may also be evaluated for their effects on T-cell dependent immunoglobulin responses and isotype switching. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Maliszewski, *J. Immunol.* 144:3028-3033, 1990; Mond, J.J. and Brunswick, M Assays for B Cell Function: *In vitro* Antibody Production, Vol 1 pp. 3.8.1-3.8.16 in Current Protocols in Immunology, J.E. Coligan *et al.* Eds., John Wiley and Sons, Toronto. 1994.

The proteins encoded by the cDNAs may also be evaluated for their effect on immune effector cells, including their effect on Th1 cells and cytotoxic lymphocytes. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Chapter 3 (*In vitro* Assays for Mouse Lymphocyte Function 3.1-3.19) and Chapter 7 (Immunologic Studies in Humans) in Current Protocols in Immunology, J.E. Coligan *et al.* Eds., Greene Publishing Associates and Wiley-Interscience; Takai *et al.*, *J. Immunol.* 137:3494-3500, 1986; Takai *et al.*, *J. Immunol.* 140:508-512, 1988; Bertagnolli *et al.*, *J. Immunol.* 149:3778-3783, 1992.

The proteins encoded by the cDNAs may also be evaluated for their effect on dendritic cell mediated activation of naive T-cells. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Guery *et al.*, *J. Immunol.* 134:536-544, 1995; Inaba *et al.*, *Journal of Experimental Medicine* 173:549-559, 1991; Macatonia *et al.*, *Journal of Immunology* 154:5071-5079, 1995; Porgador *et al.*, *Journal of Experimental Medicine* 182:255-260, 1995; Nair *et al.*, *Journal of Virology* 67:4062-4069, 1993; Huang *et al.*, *Science* 264:961-965, 1994; Macatonia *et al.*, *Journal of Experimental Medicine* 169:1255-1264, 1989; Bhardwaj *et al.*, *Journal of Clinical Investigation* 94:797-807, 1994; and Inaba *et al.*, *Journal of Experimental Medicine* 172:631-640, 1990.

The proteins encoded by the cDNAs may also be evaluated for their influence on the lifetime of lymphocytes. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Darzynkiewicz *et al.*, *Cytometry* 13:795-808, 1992; Gorczyca *et al.*, *Leukemia* 7:659-670,

1993; Gorczyca *et al.*, *Cancer Research* 53:1945-1951, 1993; Itoh *et al.*, *Cell* 66:233-243, 1991; Zacharchuk, *Journal of Immunology* 145:4037-4045, 1990; Zamai *et al.*, *Cytometry* 14:891-897, 1993; Gorczyca *et al.*, *International Journal of Oncology* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica *et al.*, *Blood* 84:111-117, 1994; Fine *et al.*, *Cellular immunology* 155:111-122, 1994; Galy *et al.*, *Blood* 85:2770-2778, 1995; Toki *et al.*, *Proc. Nat. Acad Sci. USA* 88:7548-7551, 1991.

Those proteins which exhibit activity as immune system regulators activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of immune activity is beneficial. For example, the protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, *Leishmania* spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to regulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T-cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after

exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific

tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/pr/pr mice or  
5 NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in OD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune  
10 responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory form of B lymphocyte antigens systemically.

15 Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to T cells *in vivo*, thereby  
20 activating the T cells.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to  
25 overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the  
30 surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor  
35 cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acids encoding all or a fragment of (e.g., a cytoplasmic-domain truncated fragment) of an MHC class I  $\alpha$  chain

protein and  $\beta_2$  microglobulin protein or an MHC class II  $\alpha$  chain protein and an MHC class II  $\beta$  chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class II or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject. Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

#### EXAMPLE 22

##### 15                    Assaying the Proteins Expressed from cDNAs                          or Fragments Thereof for Hematopoiesis Regulating Activity

The proteins encoded by the cDNAs or fragments thereof may also be evaluated for their hematopoiesis regulating activity. For example, the effect of the proteins on embryonic stem cell differentiation may be evaluated. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Johansson *et al.* *Cellular Biology* 15:141-151, 1995; Keller *et al.*, *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan *et al.*, *Blood* 81:2903-2915, 1993.

The proteins encoded by the cDNAs or fragments thereof may also be evaluated for their influence on the lifetime of stem cells and stem cell differentiation. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Freshney, M.G. Methylcellulose Colony Forming Assays, in Culture of Hematopoietic Cells. R.I. Freshney, *et al.* Eds. pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; McNiece, I.K. and Briddell, R.A. Primitive Hematopoietic Colony Forming Cells with High Proliferative Potential, in Culture of Hematopoietic Cells. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben *et al.*, *Experimental Hematology* 22:353-359, 1994; Ploemacher, R.E. Cobblestone Area Forming Cell Assay, In Culture of Hematopoietic Cells. R.I. Freshney, *et al.* Eds. pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Spooncer, E., Dexter, M. and Allen, T. Long Term Bone Marrow Cultures in the Presence of Stromal Cells, in Culture of Hematopoietic Cells. R.I. Freshney, *et al.* Eds. pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; and Sutherland, H.J. Long Term Culture Initiating Cell Assay, in Culture of Hematopoietic Cells. R.I. Freshney, *et al.* Eds. pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Those proteins which exhibit hematopoiesis regulatory activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of hematopoiesis is beneficial. For example, a protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even  
5 marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and  
10 proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or  
15 in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction  
20 with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy. Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

25

### EXAMPLE 23

#### Assaying the Proteins Expressed from cDNAs or Fragments Thereof for Regulation of Tissue Growth

The proteins encoded by the cDNAs or fragments thereof may also be evaluated for their effect on tissue growth. Numerous assays for such activity are familiar to those skilled in the art,  
30 including the assays disclosed in International Patent Publication No. WO95/16035, International Patent Publication No. WO95/05846 and International Patent Publication No. WO91/07491, which are incorporated herein by reference.

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, H1 and Rovee, DT, eds.), Year Book Medical  
35 Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978) which are incorporated herein by reference.

Those proteins which are involved in the regulation of tissue growth may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of tissue growth is beneficial. For example, a protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.



The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e., for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium) muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to generate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

#### EXAMPLE 24

##### Assaying the Proteins Expressed from cDNAs or Fragments

##### Thereof for Regulation of Reproductive Hormones or Cell Movement

The proteins encoded by the cDNAs or fragments thereof may also be evaluated for their ability to regulate reproductive hormones, such as follicle stimulating hormone. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Vale *et al.*, *Endocrinology* 91:562-572, 1972; Ling *et al.*, *Nature* 321:779-782, 1986; Vale *et al.*, *Nature* 321:776-779, 1986; Mason *et*

*al.*, *Nature* 318:659-663, 1985; Forage *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3091-3095, 1986. Chapter 6.12 (Measurement of Alpha and Beta Chemokines) Current Protocols in Immunology, J.E. Coligan *et al.* Eds. Greene Publishing Associates and Wiley-Interscience ; Taub *et al.* *J. Clin. Invest.* 95:1370-1376, 1995; Lind *et al.* *APMIS* 103:140-146, 1995; Muller *et al.* *Eur. J. Immunol.* 25:1744-1748; Gruber *et al.* *J. of Immunol.* 152:5860-5867, 1994; Johnston *et al.* *J. of Immunol.* 153:1762-1768, 1994.

Those proteins which exhibit activity as reproductive hormones or regulators of cell movement may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of reproductive hormones or cell movement are beneficial. For example, a protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin  $\alpha$  family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-B group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885, the disclosure of which is incorporated herein by reference. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

#### EXAMPLE 25

##### Assaying the Proteins Expressed from cDNAs or Fragments Thereof for Chemotactic/Chemokinetic Activity

The proteins encoded by the cDNAs or fragments thereof may also be evaluated for chemotactic/chemokinetic activity. For example, a protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily  
5 determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells  
10 across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub *et al.* J. Clin.  
15 Invest. 95:1370-1376, 1995; Lind *et al.* APMIS 103:140-146, 1995; Mueller *et al.* Eur. J. Immunol. 25:1744-1748; Gruber *et al.* J. of Immunol. 152:5860-5867, 1994; Johnston *et al.* J. of Immunol. 153:1762-1768, 1994.

#### EXAMPLE 26

##### Assaying the Proteins Expressed from cDNAs or 20 Fragments Thereof for Regulation of Blood Clotting

The proteins encoded by the cDNAs or fragments thereof may also be evaluated for their effects on blood clotting. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Linet *et al.*, *J. Clin. Pharmacol.* 26:131-140, 1986; Burdick *et al.*, *Thrombosis Res.*  
25 45:413-419, 1987; Humphrey *et al.*, *Fibrinolysis* 5:71-79 (1991); Schaub, *Prostaglandins* 35:467-474, 1988.

Those proteins which are involved in the regulation of blood clotting may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of blood clotting is beneficial. For example, a protein of the invention may also exhibit hemostatic or  
30 thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulations disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example,  
35 infarction of cardiac and central nervous system vessels (e.g., stroke)). Alternatively, as described in more detail below, genes encoding these proteins or nucleic acids regulating the expression of these

proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

#### EXAMPLE 27

##### Assaying the Proteins Expressed from cDNAs or Fragments Thereof for Involvement in

##### Receptor/Ligand Interactions

5 The proteins encoded by the cDNAs or a fragment thereof may also be evaluated for their involvement in receptor/ligand interactions. Numerous assays for such involvement are familiar to those skilled in the art, including the assays disclosed in the following references, which are incorporated herein by reference: Chapter 7.28 (Measurement of Cellular Adhesion under Static  
10 Conditions 7.28.1-7.28.22) in Current Protocols in Immunology, J.E. Coligan *et al.* Eds. Greene Publishing Associates and Wiley-Interscience; Takai *et al.*, *Proc. Natl. Acad. Sci. USA* 84:6864-6868, 1987; Bierer *et al.*, *J. Exp. Med.* 168:1145-1156, 1988; Rosenstein *et al.*, *J. Exp. Med.* 169:149-160, 1989; Stoltenborg *et al.*, *J. Immunol. Methods* 175:59-68, 1994; Stitt *et al.*, *Cell* 80:661-670, 1995; Gyuris *et al.*, *Cell* 75:791-803, 1993.

15 For example, the proteins of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as  
20 selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand  
25 interactions.

#### EXAMPLE 28

##### Assaying the Proteins Expressed from cDNAs or Fragments

##### Thereof for Anti-Inflammatory Activity

The proteins encoded by the cDNAs or a fragment thereof may also be evaluated for anti-  
30 inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response.  
35 Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-

reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

5

**EXAMPLE 29****Assaying the Proteins Expressed from cDNAs or Fragments Thereof for Tumor Inhibition Activity**

The proteins encoded by the cDNAs or a fragment thereof may also be evaluated for tumor inhibition activity. In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein  
10 may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote  
15 tumor growth.

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to  
20 lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting  
25 behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment  
30 of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

**EXAMPLE 30**

35

**Identification of Proteins which Interact with Polypeptides Encoded by cDNAs**

Proteins which interact with the polypeptides encoded by cDNAs or fragments thereof, such as receptor proteins, may be identified using two hybrid systems such as the Matchmaker Two

Hybrid System 2 (Catalog No. K1604-1, Clontech). As described in the manual accompanying the Matchmaker Two Hybrid System 2 (Catalog No. K1604-1, Clontech), which is incorporated herein by reference, the cDNAs or fragments thereof, are inserted into an expression vector such that they are in frame with DNA encoding the DNA binding domain of the yeast transcriptional activator GAL4. cDNAs in a cDNA library which encode proteins which might interact with the polypeptides encoded by the cDNAs or fragments thereof are inserted into a second expression vector such that they are in frame with DNA encoding the activation domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells which are positive in both the histidine selection and the lacZ assay contain plasmids encoding proteins which interact with the polypeptide encoded by the cDNAs or fragments thereof.

Alternatively, the system described in Lustig *et al.*, Methods in Enzymology 283: 83-99 (1997), the disclosure of which is incorporated herein by reference, may be used for identifying molecules which interact with the polypeptides encoded by cDNAs. In such systems, *in vitro* transcription reactions are performed on a pool of vectors containing cDNA inserts cloned downstream of a promoter which drives *in vitro* transcription. The resulting pools of mRNAs are introduced into *Xenopus laevis* oocytes. The oocytes are then assayed for a desired activity.

Alternatively, the pooled *in vitro* transcription products produced as described above may be translated *in vitro*. The pooled *in vitro* translation products can be assayed for a desired activity or for interaction with a known polypeptide.

Proteins or other molecules interacting with polypeptides encoded by cDNAs can be found by a variety of additional techniques. In one method, affinity columns containing the polypeptide encoded by the cDNA or a fragment thereof can be constructed. In some versions, of this method the affinity column contains chimeric proteins in which the protein encoded by the cDNA or a fragment thereof is fused to glutathione S-transferase. A mixture of cellular proteins or pool of expressed proteins as described above and is applied to the affinity column. Proteins interacting with the polypeptide attached to the column can then be isolated and analyzed on 2-D electrophoresis gel as described in Ramunsen *et al.* Electrophoresis, 18, 588-598 (1997), the disclosure of which is incorporated herein by reference. Alternatively, the proteins retained on the affinity column can be purified by electrophoresis based methods and sequenced. The same method can be used to isolate antibodies, to screen phage display products, or to screen phage display human antibodies.

Proteins interacting with polypeptides encoded by cDNAs or fragments thereof can also be screened by using an Optical Biosensor as described in Edwards & Leatherbarrow, Analytical Biochemistry, 246, 1-6 (1997), the disclosure of which is incorporated herein by reference. The main advantage of the method is that it allows the determination of the association rate between the

protein and other interacting molecules. Thus, it is possible to specifically select interacting molecules with a high or low association rate. Typically a target molecule is linked to the sensor surface (through a carboxymethyl dextran matrix) and a sample of test molecules is placed in contact with the target molecules. The binding of a test molecule to the target molecule causes a change in the refractive index and/ or thickness. This change is detected by the Biosensor provided it occurs in the evanescent field (which extend a few hundred nanometers from the sensor surface). In these screening assays, the target molecule can be one of the polypeptides encoded by cDNAs or a fragment thereof and the test sample can be a collection of proteins extracted from tissues or cells, a pool of expressed proteins, combinatorial peptide and/ or chemical libraries, or phage displayed peptides. The tissues or cells from which the test proteins are extracted can originate from any species.

In other methods, a target protein is immobilized and the test population is a collection of unique polypeptides encoded by the cDNAs or fragments thereof.

To study the interaction of the proteins encoded by the cDNAs or fragments thereof with drugs, the microdialysis coupled to HPLC method described by Wang *et al.*, *Chromatographia*, 44, 205-208(1997) or the affinity capillary electrophoresis method described by Busch *et al.*, *J. Chromatogr.* 777:311-328 (1997), the disclosures of which are incorporated herein by reference can be used.

The system described in U.S. Patent No. 5,654,150, the disclosure of which is incorporated herein by reference, may also be used to identify molecules which interact with the polypeptides encoded by the cDNAs. In this system, pools of cDNAs are transcribed and translated *in vitro* and the reaction products are assayed for interaction with a known polypeptide or antibody.

It will be appreciated by those skilled in the art that the proteins expressed from the cDNAs or fragments may be assayed for numerous activities in addition to those specifically enumerated above. For example, the expressed proteins may be evaluated for applications involving control and regulation of inflammation, tumor proliferation or metastasis, infection, or other clinical conditions. In addition, the proteins expressed from the cDNAs or fragments thereof may be useful as nutritional agents or cosmetic agents.

The proteins expressed from the cDNAs or fragments thereof may be used to generate antibodies capable of specifically binding to the expressed protein or fragments thereof as described below. The antibodies may be capable of binding a full length protein encoded by one of the sequences of SEQ ID NOs. 24-73, a mature protein encoded by one of the sequences of SEQ ID NOs. 24-73, or a signal peptide encoded by one of the sequences of SEQ ID Nos. 24-73. Alternatively, the antibodies may be capable of binding fragments of the proteins expressed from the cDNAs which comprise at least 10 amino acids of the sequences of SEQ ID NOs: 74-123. In some embodiments, the antibodies may be capable of binding fragments of the proteins expressed from the cDNAs which comprise at least 15 amino acids of the sequences of SEQ ID NOs: 74-123. In other

embodiments, the antibodies may be capable of binding fragments of the proteins expressed from the cDNAs which comprise at least 25 amino acids of the sequences of SEQ ID NOs: 74-123. In further embodiments, the antibodies may be capable of binding fragments of the proteins expressed from the cDNAs which comprise at least 40 amino acids of the sequences of SEQ ID NOs: 74-123.

5

### EXAMPLE 31

#### Epitopes and Antibody Fusions

A preferred embodiment of the present invention is directed to eiptope-bearing polypeptides and epitope-bearing polypeptide fragments. These epitopes may be "antigenic epitopes" or both an "antigenic epitope" and an "immunogenic epitope". An "immunogenic epitope" is defined as a part  
10 of a protein that elicits an antibody response *in vivo* when the polypeptide is the immunogen. On the other hand, a region of polypeptide to which an antibody binds is defined as an "antigenic determinant" or "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes (*See, e.g.,* Geysen, et al., 1983). It is particularly noted that although a particular epitope may not be immunogenic, it is nonetheless useful since antibodies  
15 can be made to both immunogenic and antigenic epitopes.

An epitope can comprise as few as 3 amino acids in a spatial conformation, which is unique to the epitope. Generally an epitope consists of at least 6 such amino acids, and more often at least 8-10 such amino acids. In preferred embodiment, antigenic epitopes comprise a number of amino acids that is any integer between 3 and 50. Fragments which function as epitopes may be produced  
20 by any conventional means (*See, e.g.,* Houghten, R. A., 1985), also, further described in U.S. Patent No. 4,631,211. Methods for determining the amino acids which make up an epitope include x-ray crystallography, 2-dimensional nuclear magnetic resonance, and epitope mapping, e.g., the Pepscan method described by Mario H. Geysen et al. (1984); PCT Publication No. WO 84/03564; and PCT Publication No. WO 84/03506. Epitopes may also be delineated using an algorithm, such as the  
25 algorithm of Jameson and Wolf, (Jameson and Wolf, *Comp. Appl. Biosci.* 4:181-186 (1988)). The Jameson-Wolf antigenic analysis, for example, may be performed using the computer program PROTEAN, using default parameters (Version 4.0 Windows, DNASTAR, Inc., 1228 South Park Street Madison, WI).

Table X lists antigenic peaks of predicted antigenic epitopes identified by the Jameson-Wolf  
30 algorithm. For each polypeptide referred to by its sequence identification number in the first column, the second column gives a list of antigenic peaks separated by a coma. Preferred antigenic epitopes of the present invention comprise an additional 6 amino acid residues both N-terminal and C-terminal to the positions listed in the Table. For example, for SEQ ID NO:74, the first preferred immunogenic epitope comprises amino acid residues 52 to 64. Note that for the purposes of this  
35 Table, position 1 is the N-terminal methionine residue, i.e., the leader sequence is not numbered negatively.



It is pointed out that the immunogenic epitope list describe only amino acid residues comprising epitopes predicted to have the highest degree of immunogenicity by a particular algorithm. Polypeptides of the present invention that are not specifically described as immunogenic are not considered non-antigenic. This is because they may still be antigenic *in vivo* but merely not  
5 recognized as such by the particular algorithm used. Alternatively, the polypeptides are probably antigenic *in vitro* using methods such a phage display. In fact, all fragments of the polypeptides of the present invention, at least 6 amino acids residues in length, are included in the present invention as being useful as antigenic epitope. Moreover, listed in Table IX are only the critical residues of the epitopes determined by the Jameson-Wolf analysis. Thus, additional flanking residues on either  
10 the N-terminal, C-terminal, or both N- and C-terminal ends may be added to the sequences listed to generate an epitope-bearing portion at least 6 residues in length. Amino acid residues comprising other immunogenic epitopes may be determined by algorithms similar to the Jameson-Wolf analysis or by *in vivo* testing for an antigenic response using the methods described herein or those known in the art.

15 The epitope-bearing fragments of the present invention preferably comprises 6 to 50 amino acids (i.e. any integer between 6 and 50, inclusive) of a polypeptide of the present invention. Also, included in the present invention are antigenic fragments between the integers of 6 and the full length polypeptide sequence of the sequence listing. All combinations of sequences between the integers of 6 and the full-length sequence of a polypeptide are included. The epitope-bearing  
20 fragments may be specified by either the number of contiguous amino acid residues (as a sub-genus) or by specific N-terminal and C-terminal positions (as species) as described above for the polypeptide fragments of the present invention. Any number of epitope-bearing fragments of the present invention may also be excluded in the same manner.

Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal  
25 antibodies that specifically bind the epitope (See, Wilson et al., 1984; and Sutcliffe, J. G. et al., 1983). The antibodies are then used in various techniques such as diagnostic and tissue/cell identification techniques, as described herein, and in purification methods.

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (See, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow, M. et al., (1985) and  
30 Bittle, F. J. et al., (1985)). The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.).

35 Epitope-bearing polypeptides of the present invention are used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods (See, e.g., Sutcliffe, et al., *supra*; Wilson, et al.,

*supra*, and Bittle, et al., 1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as –  
5 maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µgs of peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two  
10 weeks, to provide a useful titer of anti-peptide antibody, which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

15 As one of skill in the art will appreciate, and discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, any combination thereof including both entire domains and portions thereof) resulting in  
20 chimeric polypeptides. These fusion proteins facilitate purification, and show an increased half-life *in vivo*. This has been shown, *e.g.*, for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (*See, e.g.*, EPA 0,394,827; and Traunecker et al., 1988). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion can also be more  
25 efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone (*See, e.g.*, Fountoulakis et al., 1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag to aid in detection and purification of the expressed polypeptide.

Additional fusion proteins of the invention may be generated through the techniques of gene-  
30 shuffling, motif-shuffling, exon-shuffling, or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the present invention thereby effectively generating agonists and antagonists of the polypeptides. *See*, for example, U.S. Patent Nos.: 5,605,793; 5,811,238; 5,834,252; 5,837,458; and Patten, P.A., et al., (1997); Harayama, S., (1998); Hansson, L.O., et al (1999); and Lorenzo, M.M. and Blasco, R.,  
35 (1998). In one embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of coding polynucleotides of the invention, or the polypeptides encoded thereby may be

recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies:

The present invention further relates to antibodies and T-cell antigen receptors (TCR), which specifically bind the polypeptides, and more specifically, the epitopes of the polypeptides of the present invention. The antibodies of the present invention include IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. As used herein, the term "antibody" (Ab) is meant to include whole antibodies, including single-chain whole antibodies, and antigen binding fragments thereof. In a preferred embodiment the antibodies are human antigen binding antibody fragments of the present invention include, but are not limited to, Fab, Fab' F(ab)2 and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V<sub>L</sub> or V<sub>H</sub> domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are any combinations of variable region(s) and hinge region, CH1, CH2, and CH3 domains. The present invention further includes chimeric, humanized, and human monoclonal and polyclonal antibodies, which specifically bind the polypeptides of the present invention. The present invention further includes antibodies that are anti-idiotypic to the antibodies of the present invention.

The antibodies of the present invention may be monospecific, bispecific, and trispecific or have greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, A. et al. (1991); US Patents 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; Kostelny, S.A. et al. (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or epitope-bearing portion(s) of a polypeptide of the present invention, which are recognized or specifically bound by the antibody. In the case of proteins of the present invention secreted proteins, the antibodies may specifically bind a full-length protein encoded by a nucleic acid of the present invention, a mature protein (i.e., the protein generated by cleavage of the signal peptide) encoded by a nucleic acid of the present invention, a signal peptide encoded by a nucleic acid of the present invention, or any other polypeptide of the present invention. Therefore, the epitope(s) or epitope bearing polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or otherwise described herein (including the

sequence listing). Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded as individual species. Therefore, the present invention includes antibodies that specifically bind specified polypeptides of the present invention, and allows for the exclusion of the same.

5       Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not specifically bind any other analog, ortholog, or homolog of the polypeptides of the present invention are included. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known  
10 in the art and described herein, eg., using FASTDB and the parameters set forth herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies, which only bind polypeptides encoded by polynucleotides, which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in  
15 terms of their binding affinity. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Antibodies of the present invention have uses that include, but are not limited to, methods  
20 known in the art to purify, detect, and target the polypeptides of the present invention including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples (See, e.g., Harlow et al., 1988).

The antibodies of the present invention may be used either alone or in combination with  
25 other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. See, e.g., WO 92/08495;  
30 WO 91/14438; WO 89/12624; US Patent 5,314,995; and EP 0 396 387.

The antibodies of the present invention may be prepared by any suitable method known in the art. For example, a polypeptide of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. The term "monoclonal antibody" is not limited to antibodies produced through hybridoma  
35 technology. The term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one binding domain, where a binding domain is formed from the folding of variable domains of an antibody molecule to form three-dimensional binding spaces with an internal

surface shape and charge distribution complementary to the features of an antigenic determinant of an antigen, which allows an immunological reaction with the antigen. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared  
5 using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technology.

Hybridoma techniques include those known in the art (*See, e.g.,* Harlow et al. 1988; Hammerling, et al, 1981). (Said references incorporated by reference in their entireties). Fab and F(ab')<sub>2</sub> fragments may be produced, for example, from hybridoma-produced antibodies by  
10 proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments).

Alternatively, antibodies of the present invention can be produced through the application of recombinant DNA technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display  
15 methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle, which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 with  
20 Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman U. et al. (1995); Ames, R.S. et al. (1995); Kettleborough, C.A. et al. (1994); Persic, L. et al. (1997); Burton, D.R. et al. (1994); PCT/GB91/011134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236;  
25 WO 95/15982; WO 95/20401; and US Patents 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies,  
30 or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' F(ab)<sub>2</sub> and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax, R.L. et al. (1992); and Sawai, H. et al. (1995); and Better, M. et al. (1988).

35 Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al. (1991); Shu, L. et al. (1993); and Skerra, A. et al. (1988). For some uses, including *in vivo* use of antibodies in humans

and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. *See e.g.*, Morrison, (1985); Oi et al., (1986); Gillies, S.D. et al. (1989); and US Patent 5,807,715. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; US Patent 5,530,101; 5 and 5,585,089), veneering or resurfacing, (EP 0 592 106; EP 0 519 596; Padlan E.A., 1991; Studnicka G.M. et al., 1994; Roguska M.A. et al., 1994), and chain shuffling (US Patent 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods described above. *See also*, US Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; WO 98/46645; WO 98/50433; WO 98/24893; WO 96/34096; WO 96/33735; and WO 91/10741.

10 Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide of the present invention. The antibodies may be specific for antigens other than polypeptides of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the 15 present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *in vitro* immunoassays and purification methods using methods known in the art (*See e.g.*, Harbor et al. *supra*; WO 93/21232; EP 0 439 095; Naramura, M. et al. 1994; US Patent 5,474,981; Gillies, S.O. et al., 1992; Fell, H.P. et al., 1991).

20 The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of 25 whole domains or portions thereof. The polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the *in vivo* half-life of the polypeptides or for use in immunoassays using methods known in the art. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc 30 portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. *See e.g.*, US Patents 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388, WO 91/06570; Ashkenazi, A. et al. (1991); Zheng, X.X. et al. (1995); and Vil, H. et al. (1992).

35 The invention further relates to antibodies that act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies that disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully.

Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific antibodies, which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also include are receptor-specific antibodies which both prevent ligand binding and receptor  
5 activation. Likewise, included are neutralizing antibodies that bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies that bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies that activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be  
10 specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. The above antibody agonists can be made using methods known in the art. *See e.g.*, WO 96/40281; US Patent 5,811,097; Deng, B. et al. (1998); Chen, Z. et al. (1998); Harrop, J.A. et al. (1998); Zhu, Z. et al. (1998); Yoon, D.Y. et al. (1998); Prat, M. et al. (1998) J.; Pitard, V. et al. (1997); Liautard, J. et al. (1997); Carlson, N.G. et al. (1997) J.; Taryman, R.E. et al. (1995); Muller,  
15 Y.A. et al. (1998); Bartunek, P. et al. (1996).

As discussed above, antibodies of the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art (*See, e.g.* Greenspan and Bona (1989); and Nissinoff (1991). For example, antibodies which bind to and competitively inhibit polypeptide multimerization or  
20 binding of a polypeptide of the invention to ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization or binding domain and, as a consequence, bind to and neutralize polypeptide or its ligand. Such neutralization anti-idiotypic antibodies can be used to bind a polypeptide of the invention or to bind its ligands/receptors, and thereby block its biological activity,

25 The invention also concerns a purified or isolated antibody capable of specifically binding to a mutated full length or mature polypeptide of the present invention or to a fragment or variant thereof comprising an epitope of the mutated polypeptide. In another preferred embodiment, the present invention concerns an antibody capable of binding to a polypeptide comprising at least 10 consecutive amino acids of a polypeptide of the present invention and including at least one of the  
30 amino acids which can be encoded by the trait causing mutations.

Non-human animals or mammals, whether wild-type or transgenic, which express a different species of a polypeptide of the present invention than the one to which antibody binding is desired, and animals which do not express a polypeptide of the present invention (i.e. a knock out animal) are particularly useful for preparing antibodies. Gene knock out animals will recognize all or most of  
35 the exposed regions of a polypeptide of the present invention as foreign antigens, and therefore produce antibodies with a wider array of epitopes. Moreover, smaller polypeptides with only 10 to 30 amino acids may be useful in obtaining specific binding to any one of the polypeptides of the

present invention. In addition, the humoral immune system of animals which produce a species of a polypeptide of the present invention that resembles the antigenic sequence will preferentially recognize the differences between the animal's native polypeptide species and the antigen sequence, and produce antibodies to these unique sites in the antigen sequence. Such a technique will be particularly useful in obtaining antibodies that specifically bind to any one of the polypeptides of the present invention.

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

The antibodies of the invention may be labeled by any one of the radioactive, fluorescent or enzymatic labels known in the art.

Consequently, the invention is also directed to a method for detecting specifically the presence of a polypeptide of the present invention according to the invention in a biological sample, said method comprising the following steps:

- a) bringing into contact the biological sample with a polyclonal or monoclonal antibody that specifically binds a polypeptide of the present invention; and
- b) detecting the antigen-antibody complex formed.

The invention also concerns a diagnostic kit for detecting *in vitro* the presence of a polypeptide of the present invention in a biological sample, wherein said kit comprises:

- a) a polyclonal or monoclonal antibody that specifically binds a polypeptide of the present invention, optionally labeled;
- b) a reagent allowing the detection of the antigen-antibody complexes formed, said reagent carrying optionally a label, or being able to be recognized itself by a labeled reagent, more particularly in the case when the above-mentioned monoclonal or polyclonal antibody is not labeled by itself.

#### A. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., *Nature* 256:495 (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing



clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as Elisa, as originally described by Engvall, E., *Meth. Enzymol.* 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* Basic Methods in Molecular Biology Elsevier, New York. Section 21-2.

#### B. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.* *J. Clin. Endocrinol. Metab.* 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. *et al.*, Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12  $\mu$ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980).

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

#### V. Use of cDNAs or Fragments Thereof as Reagents

The cDNAs of the present invention may be used as reagents in isolation procedures, diagnostic assays, and forensic procedures. For example, sequences from the cDNAs (or genomic DNAs obtainable therefrom) may be detectably labeled and used as probes to isolate other sequences capable of hybridizing to them. In addition, sequences from the cDNAs (or genomic DNAs obtainable therefrom) may be used to design PCR primers to be used in isolation, diagnostic, or forensic procedures.

### Preparation of PCR Primers and Amplification of DNA

The cDNAs (or genomic DNAs obtainable therefrom) may be used to prepare PCR primers for a variety of applications, including isolation procedures for cloning nucleic acids capable of hybridizing to such sequences, diagnostic techniques and forensic techniques. The PCR primers are at least 10 bases, and preferably at least 12, 15, or 17 bases in length. More preferably, the PCR primers are at least 20-30 bases in length. In some embodiments, the PCR primers may be more than 30 bases in length. It is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see Molecular Cloning to Genetic Engineering White, B.A. Ed. in Methods in Molecular Biology 67: Humana Press, Totowa 1997. In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites.

### **EXAMPLE 33**

#### Use of cDNAs as Probes

Probes derived from cDNAs or fragments thereof (or genomic DNAs obtainable therefrom) may be labeled with detectable labels familiar to those skilled in the art, including radioisotopes and non-radioactive labels, to provide a detectable probe. The detectable probe may be single stranded or double stranded and may be made using techniques known in the art, including *in vitro* transcription, nick translation, or kinase reactions. A nucleic acid sample containing a sequence capable of hybridizing to the labeled probe is contacted with the labeled probe. If the nucleic acid in the sample is double stranded, it may be denatured prior to contacting the probe. In some applications, the nucleic acid sample may be immobilized on a surface such as a nitrocellulose or nylon membrane. The nucleic acid sample may comprise nucleic acids obtained from a variety of sources, including genomic DNA, cDNA libraries, RNA, or tissue samples.

Procedures used to detect the presence of nucleic acids capable of hybridizing to the detectable probe include well known techniques such as Southern blotting, Northern blotting, dot blotting, colony hybridization, and plaque hybridization. In some applications, the nucleic acid capable of hybridizing to the labeled probe may be cloned into vectors such as expression vectors, sequencing vectors, or *in vitro* transcription vectors to facilitate the characterization and expression of the hybridizing nucleic acids in the sample. For example, such techniques may be used to isolate and clone sequences in a genomic library or cDNA library which are capable of hybridizing to the detectable probe as described in example 17 above.

PCR primers made as described in example 32 above may be used in forensic analyses, such as the DNA fingerprinting techniques described in Examples 34-38 below. Such analyses may utilize detectable probes or primers based on the sequences of the cDNAs or fragments thereof (or genomic DNAs obtainable therefrom).

5

**EXAMPLE 34**Forensic Matching by DNA Sequencing

In one exemplary method, DNA samples are isolated from forensic specimens of, for example, hair, semen, blood or skin cells by conventional methods. A panel of PCR primers based on a number of the cDNAs (or genomic DNAs obtainable therefrom), is then utilized in accordance  
10 with example 32 to amplify DNA of approximately 100-200 bases in length from the forensic specimen. Corresponding sequences are obtained from a test subject. Each of these identification DNAs is then sequenced using standard techniques, and a simple database comparison determines the differences, if any, between the sequences from the subject and those from the sample. Statistically significant differences between the suspect's DNA sequences and those from the sample  
15 conclusively prove a lack of identity. This lack of identity can be proven, for example, with only one sequence. Identity, on the other hand, should be demonstrated with a large number of sequences, all matching. Preferably, a minimum of 50 statistically identical sequences of 100 bases in length are used to prove identity between the suspect and the sample.

**EXAMPLE 35**

20

Positive Identification by DNA Sequencing

The technique outlined in the previous example may also be used on a larger scale to provide a unique fingerprint-type identification of any individual. In this technique, primers are prepared from a large number of sequences from Table I and the appended sequence listing. Preferably, 20 to 50 different primers are used. These primers are used to obtain a corresponding  
25 number of PCR-generated DNA segments from the individual in question in accordance with example 32. Each of these DNA segments is sequenced, using the methods set forth in example 34. The database of sequences generated through this procedure uniquely identifies the individual from whom the sequences were obtained. The same panel of primers may then be used at any later time to absolutely correlate tissue or other biological specimen with that individual.

30

**EXAMPLE 36**Southern Blot Forensic Identification

The procedure of example 35 is repeated to obtain a panel of at least 10 amplified sequences from an individual and a specimen. Preferably, the panel contains at least 50 amplified sequences. More preferably, the panel contains 100 amplified sequences. In some embodiments, the panel  
35 contains 200 amplified sequences. This PCR-generated DNA is then digested with one or a combination of, preferably, four base specific restriction enzymes. Such enzymes are commercially available and known to those of skill in the art. After digestion, the resultant gene fragments are size

separated in multiple duplicate wells on an agarose gel and transferred to nitrocellulose using Southern blotting techniques well known to those with skill in the art. For a review of Southern blotting see Davis *et al.* (Basic Methods in Molecular Biology, 1986, Elsevier Press. pp 62-65).

A panel of probes based on the sequences of the cDNAs (or genomic DNAs obtainable  
5 therefrom), or fragments thereof of at least 10 bases, are radioactively or colorimetrically labeled using methods known in the art, such as nick translation or end labeling, and hybridized to the Southern blot using techniques known in the art (Davis *et al.*, supra). Preferably, the probe comprises at least 12, 15, or 17 consecutive nucleotides from the cDNA (or genomic DNAs obtainable therefrom). More preferably, the probe comprises at least 20-30 consecutive nucleotides  
10 from the cDNA (or genomic DNAs obtainable therefrom). In some embodiments, the probe comprises more than 30 nucleotides from the cDNA (or genomic DNAs obtainable therefrom). In other embodiments, the probe comprises at least 40, at least 50, at least 75, at least 100, at least 150, or at least 200 consecutive nucleotides from the cDNA (or genomic DNAs obtainable therefrom).

Preferably, at least 5 to 10 of these labeled probes are used, and more preferably at least  
15 about 20 or 30 are used to provide a unique pattern. The resultant bands appearing from the hybridization of a large sample of cDNAs (or genomic DNAs obtainable therefrom) will be a unique identifier. Since the restriction enzyme cleavage will be different for every individual, the band pattern on the Southern blot will also be unique. Increasing the number of cDNA probes will provide a statistically higher level of confidence in the identification since there will be an increased  
20 number of sets of bands used for identification.

### EXAMPLE 37

#### Dot Blot Identification Procedure

Another technique for identifying individuals using the cDNA sequences disclosed herein utilizes a dot blot hybridization technique.

25 Genomic DNA is isolated from nuclei of subject to be identified. Oligonucleotide probes of approximately 30 bp in length are synthesized that correspond to at least 10, preferably 50 sequences from the cDNAs or genomic DNAs obtainable therefrom. The probes are used to hybridize to the genomic DNA through conditions known to those in the art. The oligonucleotides are end labeled with P<sup>32</sup> using polynucleotide kinase (Pharmacia). Dot Blots are created by spotting the genomic  
30 DNA onto nitrocellulose or the like using a vacuum dot blot manifold (BioRad, Richmond California). The nitrocellulose filter containing the genomic sequences is baked or UV linked to the filter, prehybridized and hybridized with labeled probe using techniques known in the art (Davis *et al.* supra). The <sup>32</sup>P labeled DNA fragments are sequentially hybridized with successively stringent conditions to detect minimal differences between the 30 bp sequence and the DNA.  
35 Tetramethylammonium chloride is useful for identifying clones containing small numbers of nucleotide mismatches (Wood *et al.*, *Proc. Natl. Acad. Sci. USA* 82(6):1585-1588 (1985)) which is

hereby incorporated by reference. A unique pattern of dots distinguishes one individual from another individual.

cDNAs or oligonucleotides containing at least 10 consecutive bases from these sequences can be used as probes in the following alternative fingerprinting technique. Preferably, the probe  
5 comprises at least 12, 15, or 17 consecutive nucleotides from the cDNA (or genomic DNAs obtainable therefrom). More preferably, the probe comprises at least 20-30 consecutive nucleotides from the cDNA (or genomic DNAs obtainable therefrom). In some embodiments, the probe comprises more than 30 nucleotides from the cDNA (or genomic DNAs obtainable therefrom). In other embodiments, the probe comprises at least 40, at least 50, at least 75, at least 100, at least 150,  
10 or at least 200 consecutive nucleotides from the cDNA (or genomic DNAs obtainable therefrom).

Preferably, a plurality of probes having sequences from different genes are used in the alternative fingerprinting technique. Example 38 below provides a representative alternative fingerprinting procedure in which the probes are derived from cDNAs.

#### EXAMPLE 38

##### 15 Alternative "Fingerprint" Identification Technique

20-mer oligonucleotides are prepared from a large number, e.g. 50, 100, or 200, of cDNA sequences (or genomic DNAs obtainable therefrom) using commercially available oligonucleotide services such as Genset, Paris, France. Cell samples from the test subject are processed for DNA using techniques well known to those with skill in the art. The nucleic acid is digested with  
20 restriction enzymes such as EcoRI and XbaI. Following digestion, samples are applied to wells for electrophoresis. The procedure, as known in the art, may be modified to accommodate polyacrylamide electrophoresis, however in this example, samples containing 5 ug of DNA are loaded into wells and separated on 0.8% agarose gels. The gels are transferred onto nitrocellulose using standard Southern blotting techniques.

25 10 ng of each of the oligonucleotides are pooled and end-labeled with P<sup>32</sup>. The nitrocellulose is prehybridized with blocking solution and hybridized with the labeled probes. Following hybridization and washing, the nitrocellulose filter is exposed to X-Omat AR X-ray film. The resulting hybridization pattern will be unique for each individual.

It is additionally contemplated within this example that the number of probe sequences used  
30 can be varied for additional accuracy or clarity.

The antibodies generated in Examples 18 and 31 above may be used to identify the tissue type or cell species from which a sample is derived as described above.

#### EXAMPLE 39

##### Identification of Tissue Types or Cell Species by Means of Labeled Tissue Specific Antibodies

35 Identification of specific tissues is accomplished by the visualization of tissue specific antigens by means of antibody preparations according to Examples 18 and 31 which are conjugated, directly or indirectly to a detectable marker. Selected labeled antibody species bind to their specific

antigen binding partner in tissue sections, cell suspensions, or in extracts of soluble proteins from a tissue sample to provide a pattern for qualitative or semi-qualitative interpretation.

Antisera for these procedures must have a potency exceeding that of the native preparation, and for that reason, antibodies are concentrated to a mg/ml level by isolation of the gamma globulin fraction, for example, by ion-exchange chromatography or by ammonium sulfate fractionation. Also, to provide the most specific antisera, unwanted antibodies, for example to common proteins, must be removed from the gamma globulin fraction, for example by means of insoluble immunoabsorbents, before the antibodies are labeled with the marker. Either monoclonal or heterologous antisera is suitable for either procedure.

#### 10 A. Immunohistochemical Techniques

Purified, high-titer antibodies, prepared as described above, are conjugated to a detectable marker, as described, for example, by Fudenberg, H., Chap. 26 in: Basic 503 Clinical Immunology, 3rd Ed. Lange, Los Altos, California (1980) or Rose, N. *et al.*, Chap. 12 in: Methods in Immunodiagnosis, 2d Ed. John Wiley 503 Sons, New York (1980).

15 A fluorescent marker, either fluorescein or rhodamine, is preferred, but antibodies can also be labeled with an enzyme that supports a color producing reaction with a substrate, such as horseradish peroxidase. Markers can be added to tissue-bound antibody in a second step, as described below. Alternatively, the specific antitissue antibodies can be labeled with ferritin or other electron dense particles, and localization of the ferritin coupled antigen-antibody complexes  
20 achieved by means of an electron microscope. In yet another approach, the antibodies are radiolabeled, with, for example  $^{125}\text{I}$ , and detected by overlaying the antibody treated preparation with photographic emulsion.

Preparations to carry out the procedures can comprise monoclonal or polyclonal antibodies to a single protein or peptide identified as specific to a tissue type, for example, brain tissue, or  
25 antibody preparations to several antigenically distinct tissue specific antigens can be used in panels, independently or in mixtures, as required.

Tissue sections and cell suspensions are prepared for immunohistochemical examination according to common histological techniques. Multiple cryostat sections (about 4  $\mu\text{m}$ , unfixed) of the unknown tissue and known control, are mounted and each slide covered with different dilutions  
30 of the antibody preparation. Sections of known and unknown tissues should also be treated with preparations to provide a positive control, a negative control, for example, pre-immune sera, and a control for non-specific staining, for example, buffer.

Treated sections are incubated in a humid chamber for 30 min at room temperature, rinsed, then washed in buffer for 30-45 min. Excess fluid is blotted away, and the marker developed.

35 If the tissue specific antibody was not labeled in the first incubation, it can be labeled at this time in a second antibody-antibody reaction, for example, by adding fluorescein- or enzyme-

conjugated antibody against the immunoglobulin class of the antiserum-producing species, for example, fluorescein labeled antibody to mouse IgG. Such labeled sera are commercially available.

The antigen found in the tissues by the above procedure can be quantified by measuring the intensity of color or fluorescence on the tissue section, and calibrating that signal using appropriate  
5 standards.

#### B. Identification of Tissue Specific Soluble Proteins

The visualization of tissue specific proteins and identification of unknown tissues from that procedure is carried out using the labeled antibody reagents and detection strategy as described for immunohistochemistry; however the sample is prepared according to an electrophoretic technique to  
10 distribute the proteins extracted from the tissue in an orderly array on the basis of molecular weight for detection.

A tissue sample is homogenized using a Virtis apparatus; cell suspensions are disrupted by Dounce homogenization or osmotic lysis, using detergents in either case as required to disrupt cell membranes, as is the practice in the art. Insoluble cell components such as nuclei, microsomes, and  
15 membrane fragments are removed by ultracentrifugation, and the soluble protein-containing fraction concentrated if necessary and reserved for analysis.

A sample of the soluble protein solution is resolved into individual protein species by conventional SDS polyacrylamide electrophoresis as described, for example, by Davis, L. *et al.*, Section 19-2 in: Basic Methods in Molecular Biology (P. Leder, ed), Elsevier, New York (1986),  
20 using a range of amounts of polyacrylamide in a set of gels to resolve the entire molecular weight range of proteins to be detected in the sample. A size marker is run in parallel for purposes of estimating molecular weights of the constituent proteins. Sample size for analysis is a convenient volume of from 5 to 55  $\mu$ l, and containing from about 1 to 100  $\mu$ g protein. An aliquot of each of the resolved proteins is transferred by blotting to a nitrocellulose filter paper, a process that maintains  
25 the pattern of resolution. Multiple copies are prepared. The procedure, known as Western Blot Analysis, is well described in Davis, L. *et al.*, (above) Section 19-3. One set of nitrocellulose blots is stained with Coomassie Blue dye to visualize the entire set of proteins for comparison with the antibody bound proteins. The remaining nitrocellulose filters are then incubated with a solution of one or more specific antisera to tissue specific proteins prepared as described in Examples 18 and  
30 31. In this procedure, as in procedure A above, appropriate positive and negative sample and reagent controls are run.

In either procedure A or B, a detectable label can be attached to the primary tissue antigen-  
primary antibody complex according to various strategies and permutations thereof. In a straightforward approach, the primary specific antibody can be labeled; alternatively, the unlabeled  
35 complex can be bound by a labeled secondary anti-IgG antibody. In other approaches, either the primary or secondary antibody is conjugated to a biotin molecule, which can, in a subsequent step, bind an avidin conjugated marker. According to yet another strategy, enzyme labeled or radioactive

protein A, which has the property of binding to any IgG, is bound in a final step to either the primary or secondary antibody.

The visualization of tissue specific antigen binding at levels above those seen in control tissues to one or more tissue specific antibodies, prepared from the gene sequences identified from  
5 cDNA sequences, can identify tissues of unknown origin, for example, forensic samples, or differentiated tumor tissue that has metastasized to foreign bodily sites.

In addition to their applications in forensics and identification, cDNAs (or genomic DNAs obtainable therefrom) may be mapped to their chromosomal locations. example 40 below describes radiation hybrid (RH) mapping of human chromosomal regions using cDNAs. example 41 below  
10 describes a representative procedure for mapping a cDNA (or a genomic DNA obtainable therefrom) to its location on a human chromosome. example 42 below describes mapping of cDNAs (or genomic DNAs obtainable therefrom) on metaphase chromosomes by Fluorescence In Situ Hybridization (FISH).

#### EXAMPLE 40

##### 15 Radiation hybrid mapping of cDNAs to the human genome

Radiation hybrid (RH) mapping is a somatic cell genetic approach that can be used for high resolution mapping of the human genome. In this approach, cell lines containing one or more human chromosomes are lethally irradiated, breaking each chromosome into fragments whose size depends on the radiation dose. These fragments are rescued by fusion with cultured rodent cells,  
20 yielding subclones containing different fragments of the human genome. This technique is described by Benham *et al.* (*Genomics* 4:509-517, 1989) and Cox *et al.*, (*Science* 250:245-250, 1990), the entire contents of which are hereby incorporated by reference. The random and independent nature of the subclones permits efficient mapping of any human genome marker. Human DNA isolated from a panel of 80-100 cell lines provides a mapping reagent for ordering  
25 cDNAs (or genomic DNAs obtainable therefrom). In this approach, the frequency of breakage between markers is used to measure distance, allowing construction of fine resolution maps as has been done using conventional ESTs (Schuler *et al.*, *Science* 274:540-546, 1996, hereby incorporated by reference).

RH mapping has been used to generate a high-resolution whole genome radiation hybrid  
30 map of human chromosome 17q22-q25.3 across the genes for growth hormone (GH) and thymidine kinase (TK) (Foster *et al.*, *Genomics* 33:185-192, 1996), the region surrounding the Gorlin syndrome gene (Obermayr *et al.*, *Eur. J. Hum. Genet.* 4:242-245, 1996), 60 loci covering the entire short arm of chromosome 12 (Raeymaekers *et al.*, *Genomics* 29:170-178, 1995), the region of human chromosome 22 containing the neurofibromatosis type 2 locus (Frazer *et al.*, *Genomics*  
35 14:574-584, 1992) and 13 loci on the long arm of chromosome 5 (Warrington *et al.*, *Genomics* 11:701-708, 1991).

#### EXAMPLE 41



Mapping of cDNAs to Human Chromosomes using PCR techniques

cDNAs (or genomic DNAs obtainable therefrom) may be assigned to human chromosomes using PCR based methodologies. In such approaches, oligonucleotide primer pairs are designed from the cDNA sequence (or the sequence of a genomic DNA obtainable therefrom) to minimize the chance of amplifying through an intron. Preferably, the oligonucleotide primers are 18-23 bp in length and are designed for PCR amplification. The creation of PCR primers from known sequences is well known to those with skill in the art. For a review of PCR technology see Erlich, H.A., PCR Technology: Principles and Applications for DNA Amplification. 1992. W.H. Freeman and Co., New York.

10 The primers are used in polymerase chain reactions (PCR) to amplify templates from total human genomic DNA. PCR conditions are as follows: 60 ng of genomic DNA is used as a template for PCR with 80 ng of each oligonucleotide primer, 0.6 unit of Taq polymerase, and 1  $\mu$ Cu of a  $^{32}$ P-labeled deoxycytidine triphosphate. The PCR is performed in a microplate thermocycler (Techne) under the following conditions: 30 cycles of 94°C, 1.4 min; 55°C, 2 min; and 72°C, 2 min; with a final extension at 72°C for 10 min. The amplified products are analyzed on a 6% polyacrylamide sequencing gel and visualized by autoradiography. If the length of the resulting PCR product is identical to the distance between the ends of the primer sequences in the cDNA from which the primers are derived, then the PCR reaction is repeated with DNA templates from two panels of human-rodent somatic cell hybrids, BIOS PCrable DNA (BIOS Corporation) and NIGMS Human-  
15  
20 Rodent Somatic Cell Hybrid Mapping Panel Number 1 (NIGMS, Camden, NJ).

PCR is used to screen a series of somatic cell hybrid cell lines containing defined sets of human chromosomes for the presence of a given cDNA (or genomic DNA obtainable therefrom). DNA is isolated from the somatic hybrids and used as starting templates for PCR reactions using the primer pairs from the cDNAs (or genomic DNAs obtainable therefrom). Only those somatic cell hybrids with chromosomes containing the human gene corresponding to the cDNA (or genomic DNA obtainable therefrom) will yield an amplified fragment. The cDNAs (or genomic DNAs obtainable therefrom) are assigned to a chromosome by analysis of the segregation pattern of PCR products from the somatic hybrid DNA templates. The single human chromosome present in all cell hybrids that give rise to an amplified fragment is the chromosome containing that cDNA (or  
25  
30 genomic DNA obtainable therefrom). For a review of techniques and analysis of results from somatic cell gene mapping experiments. (See Ledbetter *et al.*, *Genomics* 6:475-481 (1990).)

Alternatively, the cDNAs (or genomic DNAs obtainable therefrom) may be mapped to individual chromosomes using FISH as described in example 42 below.

**EXAMPLE 42**Mapping of cDNAs to Chromosomes Using Fluorescence in situ Hybridization

35 Fluorescence in situ hybridization allows the cDNA (or genomic DNA obtainable therefrom) to be mapped to a particular location on a given chromosome. The chromosomes to be

used for fluorescence in situ hybridization techniques may be obtained from a variety of sources including cell cultures, tissues, or whole blood.

In a preferred embodiment, chromosomal localization of a cDNA (or genomic DNA obtainable therefrom) is obtained by FISH as described by Cherif *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 87:6639-6643, 1990). Metaphase chromosomes are prepared from phytohemagglutinin (PHA)-stimulated blood cell donors. PHA-stimulated lymphocytes from healthy males are cultured for 72 h in RPMI-1640 medium. For synchronization, methotrexate (10  $\mu$ M) is added for 17 h, followed by addition of 5-bromodeoxyuridine (5-BudR, 0.1 mM) for 6 h. Colcemid (1  $\mu$ g/ml) is added for the last 15 min before harvesting the cells. Cells are collected, washed in RPMI, incubated with a hypotonic solution of KCl (75 mM) at 37°C for 15 min and fixed in three changes of methanol:acetic acid (3:1). The cell suspension is dropped onto a glass slide and air dried. The cDNA (or genomic DNA obtainable therefrom) is labeled with biotin-16 dUTP by nick translation according to the manufacturer's instructions (Bethesda Research Laboratories, Bethesda, MD), purified using a Sephadex G-50 column (Pharmacia, Upssala, Sweden) and precipitated. Just prior to hybridization, the DNA pellet is dissolved in hybridization buffer (50% formamide, 2 X SSC, 10% dextran sulfate, 1 mg/ml sonicated salmon sperm DNA, pH 7) and the probe is denatured at 70°C for 5-10 min.

Slides kept at -20°C are treated for 1 h at 37°C with RNase A (100  $\mu$ g/ml), rinsed three times in 2 X SSC and dehydrated in an ethanol series. Chromosome preparations are denatured in 70% formamide, 2 X SSC for 2 min at 70°C, then dehydrated at 4°C. The slides are treated with proteinase K (10  $\mu$ g/100 ml in 20 mM Tris-HCl, 2 mM CaCl<sub>2</sub>) at 37°C for 8 min and dehydrated. The hybridization mixture containing the probe is placed on the slide, covered with a coverslip, sealed with rubber cement and incubated overnight in a humid chamber at 37°C. After hybridization and post-hybridization washes, the biotinylated probe is detected by avidin-FITC and amplified with additional layers of biotinylated goat anti-avidin and avidin-FITC. For chromosomal localization, fluorescent R-bands are obtained as previously described (Cherif *et al.*, *supra.*). The slides are observed under a LEICA fluorescence microscope (DMRXA). Chromosomes are counterstained with propidium iodide and the fluorescent signal of the probe appears as two symmetrical yellow-green spots on both chromatids of the fluorescent R-band chromosome (red). Thus, a particular cDNA (or genomic DNA obtainable therefrom) may be localized to a particular cytogenetic R-band on a given chromosome.

### EXAMPLE 43

#### Use of cDNAs to Construct or Expand Chromosome Maps

Once the cDNAs (or genomic DNAs obtainable therefrom) have been assigned to particular chromosomes using the techniques described in Examples 40-42 above, they may be utilized to construct a high resolution map of the chromosomes on which they are located or to identify the chromosomes in a sample.

Chromosome mapping involves assigning a given unique sequence to a particular chromosome as described above. Once the unique sequence has been mapped to a given chromosome, it is ordered relative to other unique sequences located on the same chromosome. One approach to chromosome mapping utilizes a series of yeast artificial chromosomes (YACs) bearing  
5 several thousand long inserts derived from the chromosomes of the organism from which the cDNAs (or genomic DNAs obtainable therefrom) are obtained. This approach is described in Ramaiah Nagaraja *et al. Genome Research* 7:210-222, March 1997. Briefly, in this approach each chromosome is broken into overlapping pieces which are inserted into the YAC vector. The YAC inserts are screened using PCR or other methods to determine whether they include the cDNA (or  
10 genomic DNA obtainable therefrom) whose position is to be determined. Once an insert has been found which includes the cDNA (or genomic DNA obtainable therefrom), the insert can be analyzed by PCR or other methods to determine whether the insert also contains other sequences known to be on the chromosome or in the region from which the cDNA (or genomic DNA obtainable therefrom) was derived. This process can be repeated for each insert in the YAC library to determine the  
15 location of each of the cDNAs (or genomic DNAs obtainable therefrom) relative to one another and to other known chromosomal markers. In this way, a high resolution map of the distribution of numerous unique markers along each of the organisms chromosomes may be obtained.

As described in example 44 below cDNAs (or genomic DNAs obtainable therefrom) may also be used to identify genes associated with a particular phenotype, such as hereditary disease or  
20 drug response.

#### EXAMPLE 44

##### Identification of genes associated with hereditary diseases or drug response

This example illustrates an approach useful for the association of cDNAs (or genomic DNAs obtainable therefrom) with particular phenotypic characteristics. In this example, a particular cDNA  
25 (or genomic DNA obtainable therefrom) is used as a test probe to associate that cDNA (or genomic DNA obtainable therefrom) with a particular phenotypic characteristic.

CDNAs (or genomic DNAs obtainable therefrom) are mapped to a particular location on a human chromosome using techniques such as those described in Examples 40 and 41 or other techniques known in the art. A search of Mendelian Inheritance in Man (V. McKusick, Mendelian  
30 Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) reveals the region of the human chromosome which contains the cDNA (or genomic DNA obtainable therefrom) to be a very gene rich region containing several known genes and several diseases or phenotypes for which genes have not been identified. The gene corresponding to this cDNA (or genomic DNA obtainable therefrom) thus becomes an immediate candidate for each of  
35 these genetic diseases.

Cells from patients with these diseases or phenotypes are isolated and expanded in culture. PCR primers from the cDNA (or genomic DNA obtainable therefrom) are used to screen genomic

DNA, mRNA or cDNA obtained from the patients. CDNAs (or genomic DNAs obtainable therefrom) that are not amplified in the patients can be positively associated with a particular disease by further analysis. Alternatively, the PCR analysis may yield fragments of different lengths when the samples are derived from an individual having the phenotype associated with the disease than  
5 when the sample is derived from a healthy individual, indicating that the gene containing the cDNA may be responsible for the genetic disease.

#### VI. Use of cDNAs (or genomic DNAs obtainable therefrom) to Construct Vectors

The present cDNAs (or genomic DNAs obtainable therefrom) may also be used to construct secretion vectors capable of directing the secretion of the proteins encoded by genes inserted in the  
10 vectors. Such secretion vectors may facilitate the purification or enrichment of the proteins encoded by genes inserted therein by reducing the number of background proteins from which the desired protein must be purified or enriched. Exemplary secretion vectors are described below.

#### **EXAMPLE 45**

##### Construction of Secretion Vectors

15 The secretion vectors of the present invention include a promoter capable of directing gene expression in the host cell, tissue, or organism of interest. Such promoters include the Rous Sarcoma Virus promoter, the SV40 promoter, the human cytomegalovirus promoter, and other promoters familiar to those skilled in the art.

A signal sequence from a cDNA (or genomic DNA obtainable therefrom), such as one of the  
20 signal sequences in SEQ ID NOs: 24-73 as defined in Table I above, is operably linked to the promoter such that the mRNA transcribed from the promoter will direct the translation of the signal peptide. The host cell, tissue, or organism may be any cell, tissue, or organism which recognizes the signal peptide encoded by the signal sequence in the cDNA (or genomic DNA obtainable therefrom). Suitable hosts include mammalian cells, tissues or organisms, avian cells, tissues, or  
25 organisms, insect cells, tissues or organisms, or yeast.

In addition, the secretion vector contains cloning sites for inserting genes encoding the proteins which are to be secreted. The cloning sites facilitate the cloning of the insert gene in frame with the signal sequence such that a fusion protein in which the signal peptide is fused to the protein encoded by the inserted gene is expressed from the mRNA transcribed from the promoter. The  
30 signal peptide directs the extracellular secretion of the fusion protein.

The secretion vector may be DNA or RNA and may integrate into the chromosome of the host, be stably maintained as an extrachromosomal replicon in the host, be an artificial chromosome, or be transiently present in the host. Preferably, the secretion vector is maintained in multiple copies in each host cell. As used herein, multiple copies means at least 2, 5, 10, 20, 25, 50 or more than 50  
35 copies per cell. In some embodiments, the multiple copies are maintained extrachromosomally. In other embodiments, the multiple copies result from amplification of a chromosomal sequence.

Many nucleic acid backbones suitable for use as secretion vectors are known to those skilled in the art, including retroviral vectors, SV40 vectors, Bovine Papilloma Virus vectors, yeast integrating plasmids, yeast episomal plasmids, yeast artificial chromosomes, human artificial chromosomes, P element vectors, baculovirus vectors, or bacterial plasmids capable of being transiently introduced  
5 into the host.

The secretion vector may also contain a polyA signal such that the polyA signal is located downstream of the gene inserted into the secretion vector.

After the gene encoding the protein for which secretion is desired is inserted into the secretion vector, the secretion vector is introduced into the host cell, tissue, or organism using  
10 calcium phosphate precipitation, DEAE-Dextran, electroporation, liposome-mediated transfection, viral particles or as naked DNA. The protein encoded by the inserted gene is then purified or enriched from the supernatant using conventional techniques such as ammonium sulfate precipitation, immunoprecipitation, immunochromatography, size exclusion chromatography, ion exchange chromatography, and hplc. Alternatively, the secreted protein may be in a sufficiently  
15 enriched or pure state in the supernatant or growth media of the host to permit it to be used for its intended purpose without further enrichment.

The signal sequences may also be inserted into vectors designed for gene therapy. In such vectors, the signal sequence is operably linked to a promoter such that mRNA transcribed from the promoter encodes the signal peptide. A cloning site is located downstream of the signal sequence  
20 such that a gene encoding a protein whose secretion is desired may readily be inserted into the vector and fused to the signal sequence. The vector is introduced into an appropriate host cell. The protein expressed from the promoter is secreted extracellularly, thereby producing a therapeutic effect.

The cDNAs or 5' ESTs may also be used to clone sequences located upstream of the cDNAs  
25 or 5' ESTs which are capable of regulating gene expression, including promoter sequences, enhancer sequences, and other upstream sequences which influence transcription or translation levels. Once identified and cloned, these upstream regulatory sequences may be used in expression vectors designed to direct the expression of an inserted gene in a desired spatial, temporal, developmental, or quantitative fashion. The next example describes a method for cloning sequences upstream of the  
30 cDNAs or 5' ESTs.

#### EXAMPLE 46

##### Use of cDNAs or Fragments thereof to Clone Upstream Sequences from Genomic DNA

Sequences derived from cDNAs or 5' ESTs may be used to isolate the promoters of the corresponding genes using chromosome walking techniques. In one chromosome walking  
35 technique, which utilizes the GenomeWalker™ kit available from Clontech, five complete genomic DNA samples are each digested with a different restriction enzyme which has a 6 base recognition

site and leaves a blunt end. Following digestion, oligonucleotide adapters are ligated to each end of the resulting genomic DNA fragments.

For each of the five genomic DNA libraries, a first PCR reaction is performed according to the manufacturer's instructions (which are incorporated herein by reference) using an outer adaptor  
5 primer provided in the kit and an outer gene specific primer. The gene specific primer should be selected to be specific for the cDNA or 5' EST of interest and should have a melting temperature, length, and location in the cDNA or 5' EST which is consistent with its use in PCR reactions. Each first PCR reaction contains 5ng of genomic DNA, 5 µl of 10X Tth reaction buffer, 0.2 mM of each dNTP, 0.2 µM each of outer adaptor primer and outer gene specific primer, 1.1 mM of Mg(OAc)<sub>2</sub>,  
10 and 1 µl of the Tth polymerase 50X mix in a total volume of 50 µl. The reaction cycle for the first PCR reaction is as follows: 1 min at 94°C / 2 sec at 94°C, 3 min at 72°C (7 cycles) / 2 sec at 94°C, 3 min at 67°C (32 cycles) / 5 min at 67°C.

The product of the first PCR reaction is diluted and used as a template for a second PCR reaction according to the manufacturer's instructions using a pair of nested primers which are  
15 located internally on the amplicon resulting from the first PCR reaction. For example, 5 µl of the reaction product of the first PCR reaction mixture may be diluted 180 times. Reactions are made in a 50 µl volume having a composition identical to that of the first PCR reaction except the nested primers are used. The first nested primer is specific for the adaptor, and is provided with the GenomeWalker™ kit. The second nested primer is specific for the particular cDNA or 5' EST for  
20 which the promoter is to be cloned and should have a melting temperature, length, and location in the cDNA or 5' EST which is consistent with its use in PCR reactions. The reaction parameters of the second PCR reaction are as follows: 1 min at 94°C / 2 sec at 94°C, 3 min at 72°C (6 cycles) / 2 sec at 94°C, 3 min at 67°C (25 cycles) / 5 min at 67°C

The product of the second PCR reaction is purified, cloned, and sequenced using standard  
25 techniques. Alternatively, two or more human genomic DNA libraries can be constructed by using two or more restriction enzymes. The digested genomic DNA is cloned into vectors which can be converted into single stranded, circular, or linear DNA. A biotinylated oligonucleotide comprising at least 15 nucleotides from the cDNA or 5' EST sequence is hybridized to the single stranded DNA. Hybrids between the biotinylated oligonucleotide and the single stranded DNA containing the cDNA  
30 or EST sequence are isolated as described in example 17 above. Thereafter, the single stranded DNA containing the cDNA or EST sequence is released from the beads and converted into double stranded DNA using a primer specific for the cDNA or 5' EST sequence or a primer corresponding to a sequence included in the cloning vector. The resulting double stranded DNA is transformed into bacteria. DNAs containing the 5' EST or cDNA sequences are identified by colony PCR or colony  
35 hybridization.

Once the upstream genomic sequences have been cloned and sequenced as described above, prospective promoters and transcription start sites within the upstream sequences may be identified

by comparing the sequences upstream of the cDNAs or 5' ESTs with databases containing known transcription start sites, transcription factor binding sites, or promoter sequences.

In addition, promoters in the upstream sequences may be identified using promoter reporter vectors as described below.

5

#### EXAMPLE 47

##### Identification of Promoters in Cloned Upstream Sequences

The genomic sequences upstream of the cDNAs or fragment thereof are cloned into a suitable promoter reporter vector, such as the pSEAP-Basic, pSEAP-Enhancer, p $\beta$ gal-Basic, p $\beta$ gal-Enhancer, or pEGFP-1 Promoter Reporter vectors available from Clontech. Briefly, each of these  
10 promoter reporter vectors include multiple cloning sites positioned upstream of a reporter gene encoding a readily assayable protein such as secreted alkaline phosphatase,  $\beta$  galactosidase, or green fluorescent protein. The sequences upstream of the cDNAs or 5' ESTs are inserted into the cloning sites upstream of the reporter gene in both orientations and introduced into an appropriate host cell. The level of reporter protein is assayed and compared to the level obtained from a vector which  
15 lacks an insert in the cloning site. The presence of an elevated expression level in the vector containing the insert with respect to the control vector indicates the presence of a promoter in the insert. If necessary, the upstream sequences can be cloned into vectors which contain an enhancer for augmenting transcription levels from weak promoter sequences. A significant level of expression above that observed with the vector lacking an insert indicates that a promoter sequence  
20 is present in the inserted upstream sequence.

Appropriate host cells for the promoter reporter vectors may be chosen based on the results of the above described determination of expression patterns of the cDNAs and ESTs. For example, if the expression pattern analysis indicates that the mRNA corresponding to a particular cDNA or fragment thereof is expressed in fibroblasts, the promoter reporter vector may be introduced into a  
25 human fibroblast cell line.

Promoter sequences within the upstream genomic DNA may be further defined by constructing nested deletions in the upstream DNA using conventional techniques such as Exonuclease III digestion. The resulting deletion fragments can be inserted into the promoter reporter vector to determine whether the deletion has reduced or obliterated promoter activity. In  
30 this way, the boundaries of the promoters may be defined. If desired, potential individual regulatory sites within the promoter may be identified using site directed mutagenesis or linker scanning to obliterate potential transcription factor binding sites within the promoter individually or in combination. The effects of these mutations on transcription levels may be determined by inserting the mutations into the cloning sites in the promoter reporter vectors.

**EXAMPLE 48**Cloning and Identification of Promoters

Using the method described in example 47 above with 5' ESTs, sequences upstream of several genes were obtained. Using the primer pairs GGG AAG ATG GAG ATA GTA TTG CCT  
5 G (SEQ ID NO:15) and CTG CCA TGT ACA TGA TAG AGA GAT TC (SEQ ID NO:16), the promoter having the internal designation P13H2 (SEQ ID NO:17) was obtained.

Using the primer pairs GTA CCA GGGG ACT GTG ACC ATT GC (SEQ ID NO:18) and CTG TGA CCA TTG CTC CCA AGA GAG (SEQ ID NO:19), the promoter having the internal designation P15B4 (SEQ ID NO:20) was obtained.

10 Using the primer pairs CTG GGA TGG AAG GCA CGG TA (SEQ ID NO:21) and GAG ACC ACA CAG CTA GAC AA (SEQ ID NO:22), the promoter having the internal designation P29B6 (SEQ ID NO:23) was obtained.

Figure 4 provides a schematic description of the promoters isolated and the way they are assembled with the corresponding 5' tags. The upstream sequences were screened for the presence  
15 of motifs resembling transcription factor binding sites or known transcription start sites using the computer program MatInspector release 2.0, August 1996.

Figure 5 describes the transcription factor binding sites present in each of these promoters. The columns labeled matrice provides the name of the MatInspector matrix used. The column labeled position provides the 5' position of the promoter site. Numeration of the sequence starts from  
20 the transcription site as determined by matching the genomic sequence with the 5' EST sequence. The column labeled "orientation" indicates the DNA strand on which the site is found, with the + strand being the coding strand as determined by matching the genomic sequence with the sequence of the 5' EST. The column labeled "score" provides the MatInspector score found for this site. The column labeled "length" provides the length of the site in nucleotides. The column labeled  
25 "sequence" provides the sequence of the site found.

The promoters and other regulatory sequences located upstream of the cDNAs or 5' ESTs may be used to design expression vectors capable of directing the expression of an inserted gene in a desired spatial, temporal, developmental, or quantitative manner. A promoter capable of directing the desired spatial, temporal, developmental, and quantitative patterns may be selected using the  
30 results of the expression analysis described in example 10 above. For example, if a promoter which confers a high level of expression in muscle is desired, the promoter sequence upstream of a cDNA or 5' EST derived from an mRNA which is expressed at a high level in muscle, as determined by the method of example 10, may be used in the expression vector.

Preferably, the desired promoter is placed near multiple restriction sites to facilitate the  
35 cloning of the desired insert downstream of the promoter, such that the promoter is able to drive expression of the inserted gene. The promoter may be inserted in conventional nucleic acid backbones designed for extrachromosomal replication, integration into the host chromosomes or



transient expression. Suitable backbones for the present expression vectors include retroviral backbones, backbones from eukaryotic episomes such as SV40 or Bovine Papilloma Virus, backbones from bacterial episomes, or artificial chromosomes.

Preferably, the expression vectors also include a polyA signal downstream of the multiple  
5 restriction sites for directing the polyadenylation of mRNA transcribed from the gene inserted into the expression vector.

Following the identification of promoter sequences using the procedures of Examples 46-48, proteins which interact with the promoter may be identified as described in example 49 below.

#### EXAMPLE 49

##### 10 Identification of Proteins Which Interact with Promoter Sequences, Upstream Regulatory Sequences, or mRNA

Sequences within the promoter region which are likely to bind transcription factors may be identified by identity to known transcription factor binding sites or through conventional mutagenesis or deletion analyses of reporter plasmids containing the promoter sequence. For  
15 example, deletions may be made in a reporter plasmid containing the promoter sequence of interest operably linked to an assayable reporter gene. The reporter plasmids carrying various deletions within the promoter region are transfected into an appropriate host cell and the effects of the deletions on expression levels is assessed. Transcription factor binding sites within the regions in which deletions reduce expression levels may be further localized using site directed mutagenesis,  
20 linker scanning analysis, or other techniques familiar to those skilled in the art. Nucleic acids encoding proteins which interact with sequences in the promoter may be identified using one-hybrid systems such as those described in the manual accompanying the Matchmaker One-Hybrid System kit available from Clontech (Catalog No. K1603-1), the disclosure of which is incorporated herein by reference. Briefly, the Matchmaker One-hybrid system is used as follows. The target sequence  
25 for which it is desired to identify binding proteins is cloned upstream of a selectable reporter gene and integrated into the yeast genome. Preferably, multiple copies of the target sequences are inserted into the reporter plasmid in tandem.

A library comprised of fusions between cDNAs to be evaluated for the ability to bind to the promoter and the activation domain of a yeast transcription factor, such as GAL4, is transformed  
30 into the yeast strain containing the integrated reporter sequence. The yeast are plated on selective media to select cells expressing the selectable marker linked to the promoter sequence. The colonies which grow on the selective media contain genes encoding proteins which bind the target sequence. The inserts in the genes encoding the fusion proteins are further characterized by sequencing. In addition, the inserts may be inserted into expression vectors or *in vitro* transcription vectors.  
35 Binding of the polypeptides encoded by the inserts to the promoter DNA may be confirmed by techniques familiar to those skilled in the art, such as gel shift analysis or DNase protection analysis.

## VII. Use of cDNAs (or Genomic DNAs Obtainable Therefrom) in Gene Therapy

The present invention also comprises the use of cDNAs (or genomic DNAs obtainable therefrom) in gene therapy strategies, including antisense and triple helix strategies as described in Examples 50 and 51 below. In antisense approaches, nucleic acid sequences complementary to an mRNA are hybridized to the mRNA intracellularly, thereby blocking the expression of the protein encoded by the mRNA. The antisense sequences may prevent gene expression through a variety of mechanisms. For example, the antisense sequences may inhibit the ability of ribosomes to translate the mRNA. Alternatively, the antisense sequences may block transport of the mRNA from the nucleus to the cytoplasm, thereby limiting the amount of mRNA available for translation. Another mechanism through which antisense sequences may inhibit gene expression is by interfering with mRNA splicing. In yet another strategy, the antisense nucleic acid may be incorporated in a ribozyme capable of specifically cleaving the target mRNA.

### **EXAMPLE 50**

#### Preparation and Use of Antisense Oligonucleotides

The antisense nucleic acid molecules to be used in gene therapy may be either DNA or RNA sequences. They may comprise a sequence complementary to the sequence of the cDNA (or genomic DNA obtainable therefrom). The antisense nucleic acids should have a length and melting temperature sufficient to permit formation of an intracellular duplex having sufficient stability to inhibit the expression of the mRNA in the duplex. Strategies for designing antisense nucleic acids suitable for use in gene therapy are disclosed in Green *et al.*, *Ann. Rev. Biochem.*, 55:569-597 (1986) and Izant and Weintraub, *Cell*, 36:1007-1015 (1984), which are hereby incorporated by reference.

In some strategies, antisense molecules are obtained from a nucleotide sequence encoding a protein by reversing the orientation of the coding region with respect to a promoter so as to transcribe the opposite strand from that which is normally transcribed in the cell. The antisense molecules may be transcribed using *in vitro* transcription systems such as those which employ T7 or SP6 polymerase to generate the transcript. Another approach involves transcription of the antisense nucleic acids *in vivo* by operably linking DNA containing the antisense sequence to a promoter in an expression vector.

Alternatively, oligonucleotides which are complementary to the strand normally transcribed in the cell may be synthesized *in vitro*. Thus, the antisense nucleic acids are complementary to the corresponding mRNA and are capable of hybridizing to the mRNA to create a duplex. In some embodiments, the antisense sequences may contain modified sugar phosphate backbones to increase stability and make them less sensitive to RNase activity. Examples of modifications suitable for use in antisense strategies include 2' O-methyl RNA oligonucleotides and Protein-nucleic acid (PNA) oligonucleotides. Further examples are described by Rossi *et al.*, *Pharmacol. Ther.*, 50(2):245-254, (1991).

Various types of antisense oligonucleotides complementary to the sequence of the cDNA (or genomic DNA obtainable therefrom) may be used. In one preferred embodiment, stable and semi-stable antisense oligonucleotides described in International Application No. PCT WO94/23026, hereby incorporated by reference, are used. In these molecules, the 3' end or both the 3' and 5' ends  
5 are engaged in intramolecular hydrogen bonding between complementary base pairs. These molecules are better able to withstand exonuclease attacks and exhibit increased stability compared to conventional antisense oligonucleotides.

In another preferred embodiment, the antisense oligodeoxynucleotides against herpes simplex virus types 1 and 2 described in International Application No. WO 95/04141, hereby  
10 incorporated by reference, are used.

In yet another preferred embodiment, the covalently cross-linked antisense oligonucleotides described in International Application No. WO 96/31523, hereby incorporated by reference, are used. These double- or single-stranded oligonucleotides comprise one or more, respectively, inter- or intra-oligonucleotide covalent cross-linkages, wherein the linkage consists of an amide bond  
15 between a primary amine group of one strand and a carboxyl group of the other strand or of the same strand, respectively, the primary amine group being directly substituted in the 2' position of the strand nucleotide monosaccharide ring, and the carboxyl group being carried by an aliphatic spacer group substituted on a nucleotide or nucleotide analog of the other strand or the same strand, respectively.

The antisense oligodeoxynucleotides and oligonucleotides disclosed in International Application No. WO 92/18522, incorporated by reference, may also be used. These molecules are stable to degradation and contain at least one transcription control recognition sequence which binds to control proteins and are effective as decoys therefor. These molecules may contain "hairpin" structures, "dumbbell" structures, "modified dumbbell" structures, "cross-linked" decoy structures  
20 and "loop" structures.  
25

In another preferred embodiment, the cyclic double-stranded oligonucleotides described in European Patent Application No. 0 572 287 A2, hereby incorporated by reference are used. These ligated oligonucleotide "dumbbells" contain the binding site for a transcription factor and inhibit expression of the gene under control of the transcription factor by sequestering the factor.

Use of the closed antisense oligonucleotides disclosed in International Application No. WO 92/19732, hereby incorporated by reference, is also contemplated. Because these molecules have no free ends, they are more resistant to degradation by exonucleases than are conventional oligonucleotides. These oligonucleotides may be multifunctional, interacting with several regions which are not adjacent to the target mRNA.

The appropriate level of antisense nucleic acids required to inhibit gene expression may be  
35 determined using *in vitro* expression analysis. The antisense molecule may be introduced into the cells by diffusion, injection, infection or transfection using procedures known in the art. For

example, the antisense nucleic acids can be introduced into the body as a bare or naked oligonucleotide, oligonucleotide encapsulated in lipid, oligonucleotide sequence encapsulated by viral protein, or as an oligonucleotide operably linked to a promoter contained in an expression vector. The expression vector may be any of a variety of expression vectors known in the art, including retroviral or viral vectors, vectors capable of extrachromosomal replication, or integrating vectors. The vectors may be DNA or RNA.

The antisense molecules are introduced onto cell samples at a number of different concentrations preferably between  $1 \times 10^{-10} \text{M}$  to  $1 \times 10^{-4} \text{M}$ . Once the minimum concentration that can adequately control gene expression is identified, the optimized dose is translated into a dosage suitable for use *in vivo*. For example, an inhibiting concentration in culture of  $1 \times 10^{-7}$  translates into a dose of approximately 0.6 mg/kg bodyweight. Levels of oligonucleotide approaching 100 mg/kg bodyweight or higher may be possible after testing the toxicity of the oligonucleotide in laboratory animals. It is additionally contemplated that cells from the vertebrate are removed, treated with the antisense oligonucleotide, and reintroduced into the vertebrate.

It is further contemplated that the antisense oligonucleotide sequence is incorporated into a ribozyme sequence to enable the antisense to specifically bind and cleave its target mRNA. For technical applications of ribozyme and antisense oligonucleotides see Rossi *et al.*, *supra*.

In a preferred application of this invention, the polypeptide encoded by the gene is first identified, so that the effectiveness of antisense inhibition on translation can be monitored using techniques that include but are not limited to antibody-mediated tests such as RIAs and ELISA, functional assays, or radiolabeling.

The cDNAs of the present invention (or genomic DNAs obtainable therefrom) may also be used in gene therapy approaches based on intracellular triple helix formation. Triple helix oligonucleotides are used to inhibit transcription from a genome. They are particularly useful for studying alterations in cell activity as it is associated with a particular gene. The cDNAs (or genomic DNAs obtainable therefrom) of the present invention or, more preferably, a fragment of those sequences, can be used to inhibit gene expression in individuals having diseases associated with expression of a particular gene. Similarly, a fragment of the cDNA (or genomic DNA obtainable therefrom) can be used to study the effect of inhibiting transcription of a particular gene within a cell. Traditionally, homopurine sequences were considered the most useful for triple helix strategies. However, homopyrimidine sequences can also inhibit gene expression. Such homopyrimidine oligonucleotides bind to the major groove at homopurine:homopyrimidine sequences. Thus, both types of sequences from the cDNA or from the gene corresponding to the cDNA are contemplated within the scope of this invention.

**EXAMPLE 51****Preparation and use of Triple Helix Probes**

The sequences of the cDNAs (or genomic DNAs obtainable therefrom) are scanned to identify 10-mer to 20-mer homopyrimidine or homopurine stretches which could be used in triple-helix based strategies for inhibiting gene expression. Following identification of candidate homopyrimidine or homopurine stretches, their efficiency in inhibiting gene expression is assessed by introducing varying amounts of oligonucleotides containing the candidate sequences into tissue culture cells which normally express the target gene. The oligonucleotides may be prepared on an oligonucleotide synthesizer or they may be purchased commercially from a company specializing in custom oligonucleotide synthesis, such as GENSET, Paris, France.

The oligonucleotides may be introduced into the cells using a variety of methods known to those skilled in the art, including but not limited to calcium phosphate precipitation, DEAE-Dextran, electroporation, liposome-mediated transfection or native uptake.

Treated cells are monitored for altered cell function or reduced gene expression using techniques such as Northern blotting, RNase protection assays, or PCR based strategies to monitor the transcription levels of the target gene in cells which have been treated with the oligonucleotide. The cell functions to be monitored are predicted based upon the homologies of the target gene corresponding to the cDNA from which the oligonucleotide was derived with known gene sequences that have been associated with a particular function. The cell functions can also be predicted based on the presence of abnormal physiologies within cells derived from individuals with a particular inherited disease, particularly when the cDNA is associated with the disease using techniques described in example 44.

The oligonucleotides which are effective in inhibiting gene expression in tissue culture cells may then be introduced *in vivo* using the techniques described above and in example 50 at a dosage calculated based on the *in vitro* results, as described in example 50.

In some embodiments, the natural (beta) anomers of the oligonucleotide units can be replaced with alpha anomers to render the oligonucleotide more resistant to nucleases. Further, an intercalating agent such as ethidium bromide, or the like, can be attached to the 3' end of the alpha oligonucleotide to stabilize the triple helix. For information on the generation of oligonucleotides suitable for triple helix formation see Griffin *et al.* (*Science*, 245:967-971 (1989), which is hereby incorporated by this reference).

**EXAMPLE 52****Use of cDNAs to Express an Encoded Protein in a Host Organism**

The cDNAs of the present invention may also be used to express an encoded protein in a host organism to produce a beneficial effect. In such procedures, the encoded protein may be transiently expressed in the host organism or stably expressed in the host organism. The encoded protein may have any of the activities described above. The encoded protein may be a protein which

the host organism lacks or, alternatively, the encoded protein may augment the existing levels of the protein in the host organism.

A full length cDNA encoding the signal peptide and the mature protein, or a cDNA encoding only the mature protein is introduced into the host organism. The cDNA may be introduced into the host organism using a variety of techniques known to those of skill in the art. For example, the cDNA may be injected into the host organism as naked DNA such that the encoded protein is expressed in the host organism, thereby producing a beneficial effect.

Alternatively, the cDNA may be cloned into an expression vector downstream of a promoter which is active in the host organism. The expression vector may be any of the expression vectors designed for use in gene therapy, including viral or retroviral vectors.

The expression vector may be directly introduced into the host organism such that the encoded protein is expressed in the host organism to produce a beneficial effect. In another approach, the expression vector may be introduced into cells *in vitro*. Cells containing the expression vector are thereafter selected and introduced into the host organism, where they express the encoded protein to produce a beneficial effect.

### EXAMPLE 53

#### Use Of Signal Peptides To Import Proteins Into Cells

The short core hydrophobic region (h) of signal peptides encoded by the cDNAs of the present invention or fragment thereof may also be used as a carrier to import a peptide or a protein of interest, so-called cargo, into tissue culture cells (Lin *et al.*, *J. Biol. Chem.*, 270: 14225-14258 (1995); Du *et al.*, *J. Peptide Res.*, 51: 235-243 (1998); Rojas *et al.*, *Nature Biotech.*, 16: 370-375 (1998)).

When cell permeable peptides of limited size (approximately up to 25 amino acids) are to be translocated across cell membrane, chemical synthesis may be used in order to add the h region to either the C-terminus or the N-terminus to the cargo peptide of interest. Alternatively, when longer peptides or proteins are to be imported into cells, nucleic acids can be genetically engineered, using techniques familiar to those skilled in the art, in order to link the cDNA sequence or fragment thereof encoding the h region to the 5' or the 3' end of a DNA sequence coding for a cargo polypeptide. Such genetically engineered nucleic acids are then translated either *in vitro* or *in vivo* after transfection into appropriate cells, using conventional techniques to produce the resulting cell permeable polypeptide. Suitable hosts cells are then simply incubated with the cell permeable polypeptide which is then translocated across the membrane.

This method may be applied to study diverse intracellular functions and cellular processes. For instance, it has been used to probe functionally relevant domains of intracellular proteins and to examine protein-protein interactions involved in signal transduction pathways (Lin *et al.*, *supra*; Lin *et al.*, *J. Biol. Chem.*, 271: 5305-5308 (1996); Rojas *et al.*, *J. Biol. Chem.*, 271: 27456-27461 (1996);

Liu *et al.*, *Proc. Natl. Acad. Sci. USA*, 93: 11819-11824 (1996); Rojas *et al.*, *Bioch. Biophys. Res. Commun.*, 234: 675-680 (1997)).

Such techniques may be used in cellular therapy to import proteins producing therapeutic effects. For instance, cells isolated from a patient may be treated with imported therapeutic proteins  
5 and then re-introduced into the host organism.

Alternatively, the h region of signal peptides of the present invention could be used in combination with a nuclear localization signal to deliver nucleic acids into cell nucleus. Such oligonucleotides may be antisense oligonucleotides or oligonucleotides designed to form triple helixes, as described in examples 50 and 51 respectively, in order to inhibit processing and  
10 maturation of a target cellular RNA.

#### **EXAMPLE 54**

##### **Computer Embodiments**

As used herein the term "cDNA codes of SEQ ID NOs. 24-73" encompasses the nucleotide sequences of SEQ ID NOs. 24-73, fragments of SEQ ID NOs. 24-73, nucleotide sequences  
15 homologous to SEQ ID NOs. 24-73 or homologous to fragments of SEQ ID NOs. 24-73, and sequences complementary to all of the preceding sequences. The fragments include fragments of SEQ ID NOs. 24-73 comprising at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 consecutive nucleotides of SEQ ID NOs. 24-73. Preferably, the fragments are novel fragments. Preferably the fragments include polynucleotides described in Table  
20 III or fragments thereof comprising at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 consecutive nucleotides of the polynucleotides described in Table III. Homologous sequences and fragments of SEQ ID NOs. 24-73 refer to a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% identity to these sequences. Identity may be determined using any of the computer programs and parameters described in example 17, including  
25 BLAST2N with the default parameters or with any modified parameters. Homologous sequences also include RNA sequences in which uridines replace the thymines in the cDNA codes of SEQ ID NOs. 24-73. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error as described above. Preferably the homologous sequences and fragments of SEQ ID NOs. 24-73 include polynucleotides described in  
30 Table III or fragments comprising at least 8, 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 1000 or 2000 consecutive nucleotides of the polynucleotides described in Table III. It will be appreciated that the cDNA codes of SEQ ID NOs. 24-73 can be represented in the traditional single character format (See the inside back cover of Styer, Lubert. *Biochemistry*, 3<sup>rd</sup> edition. W. H Freeman & Co., New York.) or in any other format which records the identity of the  
35 nucleotides in a sequence.

As used herein the term "polypeptide codes of SEQ ID NOS. 74-123" encompasses the polypeptide sequences of SEQ ID NOS. 74-123 which are encoded by the cDNAs of SEQ ID NOS.

24-73, polypeptide sequences homologous to the polypeptides of SEQ ID NOS. 74-123, or fragments of any of the preceding sequences. Homologous polypeptide sequences refer to a polypeptide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75% identity to one of the polypeptide sequences of SEQ ID NOS. 74-123. Identity may be determined using any of the computer programs and parameters described herein, including FASTA with the default parameters or with any modified parameters. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error as described above. The polypeptide fragments comprise at least 5, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 150 or 200 consecutive amino acids of the polypeptides of SEQ ID NOS. 74-123. Preferably, the fragments are novel fragments. Preferably, the fragments include polypeptides encoded by the polynucleotides described in Table III, or fragments thereof comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of the polypeptides encoded by the polynucleotides described in Table III. It will be appreciated that the polypeptide codes of the SEQ ID NOS. 74-123 can be represented in the traditional single character format or three letter format (See the inside back cover of Starrier, Lubert. *Biochemistry*, 3<sup>rd</sup> edition. W. H Freeman & Co., New York.) or in any other format which relates the identity of the polypeptides in a sequence.

It will be appreciated by those skilled in the art that the cDNA codes of SEQ ID NOS. 24-73 and polypeptide codes of SEQ ID NOS. 74-123 can be stored, recorded, and manipulated on any medium which can be read and accessed by a computer. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any of the presently known methods for recording information on a computer readable medium to generate manufactures comprising one or more of the cDNA codes of SEQ ID NOS. 24-73, one or more of the polypeptide codes of SEQ ID NOS. 74-123. Another aspect of the present invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, 20, 25, 30, or 50 cDNA codes of SEQ ID NOS. 24-73. Another aspect of the present invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, 20, 25, 30, or 50 polypeptide codes of SEQ ID NOS. 74-123.

Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disk, a floppy disk, a magnetic tape, CD-ROM, Digital Versatile Disk (DVD), Random Access Memory (RAM), or Read Only Memory (ROM) as well as other types of other media known to those skilled in the art.

Embodiments of the present invention include systems, particularly computer systems which store and manipulate the sequence information described herein. One example of a computer system is illustrated in block diagram form in Figure 6. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze the nucleotide sequences of the cDNA codes of SEQ ID NOS. 24-73, or the amino acid sequences of the



polypeptide codes of SEQ ID NOS. 74-123. In one embodiment, the computer system 100 is a Sun Enterprise 1000 server (Sun Microsystems, Palo Alto, CA). The computer system 100 preferably includes a processor for processing, accessing and manipulating the sequence data. The processor 105 can be any well-known type of central processing unit, such as the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq or International Business Machines.

Preferably, the computer system 100 is a general purpose system that comprises the processor 105 and one or more internal data storage components 110 for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

10 In one particular embodiment, the computer system 100 includes a processor 105 connected to a bus which is connected to a main memory 115 (preferably implemented as RAM) and one or more internal data storage devices 110, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system 100 further includes one or more data retrieving device 118 for reading the data stored on the internal data storage devices 15 110.

The data retrieving device 118 may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, etc. In some embodiments, the internal data storage device 110 is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system 100 may 20 advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

The computer system 100 includes a display 120 which is used to display output to a computer user. It should also be noted that the computer system 100 can be linked to other computer systems 125a-c in a network or wide area network to provide centralized access to the computer 25 system 100.

Software for accessing and processing the nucleotide sequences of the cDNA codes of SEQ ID NOS. 24-73, or the amino acid sequences of the polypeptide codes of SEQ ID NOS. 74-123 (such as search tools, compare tools, and modeling tools etc.) may reside in main memory 115 during execution.

30 In some embodiments, the computer system 100 may further comprise a sequence comparer for comparing the above-described cDNA codes of SEQ ID NOS. 24-73 or polypeptide codes of SEQ ID NOS. 74-123 stored on a computer readable medium to reference nucleotide or polypeptide sequences stored on a computer readable medium. A "sequence comparer" refers to one or more programs which are implemented on the computer system 100 to compare a nucleotide or 35 polypeptide sequence with other nucleotide or polypeptide sequences and/or compounds including but not limited to peptides, peptidomimetics, and chemicals stored within the data storage means. For example, the sequence comparer may compare the nucleotide sequences of the cDNA codes of

SEQ ID NOS. 24-73, or the amino acid sequences of the polypeptide codes of SEQ ID NOS. 74-123 stored on a computer readable medium to reference sequences stored on a computer readable medium to identify homologies, motifs implicated in biological function, or structural motifs. The various sequence comparer programs identified elsewhere in this patent specification are particularly  
5 contemplated for use in this aspect of the invention.

Figure 7 is a flow diagram illustrating one embodiment of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the identity levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system 100, or a public database such as  
10 GENBANK, PIR or SWISSPROT that is available through the Internet.

The process 200 begins at a start state 201 and then moves to a state 202 wherein the new sequence to be compared is stored to a memory in a computer system 100. As discussed above, the memory could be any type of memory, including RAM or an internal storage device.

The process 200 then moves to a state 204 wherein a database of sequences is opened for  
15 analysis and comparison. The process 200 then moves to a state 206 wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state 210 to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for  
20 comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the identity level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system.

Once a comparison of the two sequences has been performed at the state 210, a  
25 determination is made at a decision state 210 whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the identity parameters entered by the user will be marked as "same" in the process 200.

If a determination is made that the two sequences are the same, the process 200 moves to a state 214 wherein the name of the sequence from the database is displayed to the user. This state  
30 notifies the user that the sequence with the displayed name fulfills the identity constraints that were entered. Once the name of the stored sequence is displayed to the user, the process 200 moves to a decision state 218 wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process 200 terminates at an end state 220. However, if more sequences do exist in the database, then the process 200 moves to a state 224  
35 wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database.

It should be noted that if a determination had been made at the decision state 212 that the sequences were not homologous, then the process 200 would move immediately to the decision state 218 in order to determine if any other sequences were available in the database for comparison.

Accordingly, one aspect of the present invention is a computer system comprising a processor, a data storage device having stored thereon a nucleic acid code of SEQ ID NOS. 24-73 or a polypeptide code of SEQ ID NOS. 74-123, a data storage device having retrievably stored thereon reference nucleotide sequences or polypeptide sequences to be compared to the nucleic acid code of SEQ ID NOS. 24-73 or polypeptide code of SEQ ID NOS. 74-123 and a sequence comparer for conducting the comparison. The sequence comparer may indicate a identity level between the sequences compared or identify structural motifs in the above described nucleic acid code of SEQ ID NOS. 24-73 and polypeptide codes of SEQ ID NOS. 74-123 or it may identify structural motifs in sequences which are compared to these cDNA codes and polypeptide codes. In some embodiments, the data storage device may have stored thereon the sequences of at least 2, 5, 10, 15, 20, 25, 30, or 50 of the cDNA codes of SEQ ID NOS. 24-73 or polypeptide codes of SEQ ID NOS. 74-123.

Another aspect of the present invention is a method for determining the level of identity between a nucleic acid code of SEQ ID NOS. 24-73 and a reference nucleotide sequence, comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through the use of a computer program which determines identity levels and determining identity between the nucleic acid code and the reference nucleotide sequence with the computer program. The computer program may be any of a number of computer programs for determining identity levels, including those specifically enumerated herein, including BLAST2N with the default parameters or with any modified parameters. The method may be implemented using the computer systems described above. The method may also be performed by reading 2, 5, 10, 15, 20, 25, 30, or 50 of the above described cDNA codes of SEQ ID NOS. 24-73 through use of the computer program and determining identity between the cDNA codes and reference nucleotide sequences.

Figure 8 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous. The process 250 begins at a start state 252 and then moves to a state 254 wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state 256. The process 250 then moves to a state 260 wherein the first character in the first sequence is read and then to a state 262 wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it should be in the single letter amino acid code so that the first and second sequences can be easily compared.

A determination is then made at a decision state 264 whether the two characters are the same. If they are the same, then the process 250 moves to a state 268 wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters

are the same. If they are, then the process 250 continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process 250 moves to a decision state 274 to determine whether there are any more characters either sequence to read.

5        If there aren't any more characters to read, then the process 250 moves to a state 276 wherein the level of identity between the first and second sequences is displayed to the user. The level of identity is determined by calculating the profragment of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the  
10    identity level would be 100%.

         Alternatively, the computer program may be a computer program which compares the nucleotide sequences of the cDNA codes of the present invention, to reference nucleotide sequences in order to determine whether the nucleic acid code of SEQ ID NOs. 24-73 differs from a reference nucleic acid sequence at one or more positions. Optionally such a program records the length and  
15    identity of inserted, deleted or substituted nucleotides with respect to the sequence of either the reference polynucleotide or the nucleic acid code of SEQ ID NOs. 24-73. In one embodiment, the computer program may be a program which determines whether the nucleotide sequences of the cDNA codes of SEQ ID NOs. 24-73 contain a biallelic marker or single nucleotide polymorphism (SNP) with respect to a reference nucleotide sequence. This single nucleotide polymorphism may  
20    comprise a single base substitution, insertion, or deletion, while this biallelic marker may comprise about one to ten consecutive bases substituted, inserted or deleted.

         Another aspect of the present invention is a method for determining the level of identity between a polypeptide code of SEQ ID NOS. 74-123 and a reference polypeptide sequence, comprising the steps of reading the polypeptide code of SEQ ID NOS. 74-123 and the reference  
25    polypeptide sequence through use of a computer program which determines identity levels and determining identity between the polypeptide code and the reference polypeptide sequence using the computer program.

         Accordingly, another aspect of the present invention is a method for determining whether a nucleic acid code of SEQ ID NOs. 24-73 differs at one or more nucleotides from a reference  
30    nucleotide sequence comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through use of a computer program which identifies differences between nucleic acid sequences and identifying differences between the nucleic acid code and the reference nucleotide sequence with the computer program. In some embodiments, the computer program is a program which identifies single nucleotide polymorphisms. The method may be implemented by  
35    the computer systems described above and the method illustrated in Figure 8. The method may also be performed by reading at least 2, 5, 10, 15, 20, 25, 30, or 50 of the cDNA codes of SEQ ID NOs. 24-73 and the reference nucleotide sequences through the use of the computer program and

identifying differences between the cDNA codes and the reference nucleotide sequences with the computer program.

In other embodiments the computer based system may further comprise an identifier for identifying features within the nucleotide sequences of the cDNA codes of SEQ ID NOS. 24-73 or  
5 the amino acid sequences of the polypeptide codes of SEQ ID NOS. 74-123.

An "identifier" refers to one or more programs which identifies certain features within the above-described nucleotide sequences of the cDNA codes of SEQ ID NOS. 24-73 or the amino acid sequences of the polypeptide codes of SEQ ID NOS. 74-123. In one embodiment, the identifier may comprise a program which identifies an open reading frame in the cDNAs codes of SEQ ID NOS.  
10 24-73.

Figure 9 is a flow diagram illustrating one embodiment of an identifier process 300 for detecting the presence of a feature in a sequence. The process 300 begins at a start state 302 and then moves to a state 304 wherein a first sequence that is to be checked for features is stored to a memory 115 in the computer system 100. The process 300 then moves to a state 306 wherein a  
15 database of sequence features is opened. Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG". Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group ([www.gcg.com](http://www.gcg.com)).

20 Once the database of features is opened at the state 306, the process 300 moves to a state 308 wherein the first feature is read from the database. A comparison of the attribute of the first feature with the first sequence is then made at a state 310. A determination is then made at a decision state 316 whether the attribute of the feature was found in the first sequence. If the attribute was found, then the process 300 moves to a state 318 wherein the name of the found feature is  
25 displayed to the user.

The process 300 then moves to a decision state 320 wherein a determination is made whether more features exist in the database. If no more features do exist, then the process 300 terminates at an end state 324. However, if more features do exist in the database, then the process 300 reads the next sequence feature at a state 326 and loops back to the state 310 wherein the  
30 attribute of the next feature is compared against the first sequence.

It should be noted, that if the feature attribute is not found in the first sequence at the decision state 316, the process 300 moves directly to the decision state 320 in order to determine if any more features exist in the database.

In another embodiment, the identifier may comprise a molecular modeling program which  
35 determines the 3-dimensional structure of the polypeptides codes of SEQ ID NOS. 74-123. In some embodiments, the molecular modeling program identifies target sequences that are most compatible with profiles representing the structural environments of the residues in known three-dimensional

protein structures. (See, e.g., Eisenberg *et al.*, U.S. Patent No. 5,436,850 issued July 25, 1995). In another technique, the known three-dimensional structures of proteins in a given family are superimposed to define the structurally conserved regions in that family. This protein modeling technique also uses the known three-dimensional structure of a homologous protein to approximate the structure of the polypeptide codes of SEQ ID NOS. 74-123. (See e.g., Srinivasan, *et al.*, U.S. Patent No. 5,557,535 issued September 17, 1996). Conventional identity modeling techniques have been used routinely to build models of proteases and antibodies. (Sowdhamini *et al.*, Protein Engineering 10:207, 215 (1997)). Comparative approaches can also be used to develop three-dimensional protein models when the protein of interest has poor sequence identity to template proteins. In some cases, proteins fold into similar three-dimensional structures despite having very weak sequence identities. For example, the three-dimensional structures of a number of helical cytokines fold in similar three-dimensional topology in spite of weak sequence identity.

The recent development of threading methods now enables the identification of likely folding patterns in a number of situations where the structural relatedness between target and template(s) is not detectable at the sequence level. Hybrid methods, in which fold recognition is performed using Multiple Sequence Threading (MST), structural equivalencies are deduced from the threading output using a distance geometry program DRAGON to construct a low resolution model, and a full-atom representation is constructed using a molecular modeling package such as QUANTA.

According to this 3-step approach, candidate templates are first identified by using the novel fold recognition algorithm MST, which is capable of performing simultaneous threading of multiple aligned sequences onto one or more 3-D structures. In a second step, the structural equivalencies obtained from the MST output are converted into inter-residue distance restraints and fed into the distance geometry program DRAGON, together with auxiliary information obtained from secondary structure predictions. The program combines the restraints in an unbiased manner and rapidly generates a large number of low resolution model confirmations. In a third step, these low resolution model confirmations are converted into full-atom models and subjected to energy minimization using the molecular modeling package QUANTA. (See e.g., Aszodi *et al.*, Proteins:Structure, Function, and Genetics, Supplement 1:38-42 (1997)).

The results of the molecular modeling analysis may then be used in rational drug design techniques to identify agents which modulate the activity of the polypeptide codes of SEQ ID NOS. 74-123.

Accordingly, another aspect of the present invention is a method of identifying a feature within the cDNA codes of SEQ ID NOS. 24-73 or the polypeptide codes of SEQ ID NOS. 74-123 comprising reading the nucleic acid code(s) or the polypeptide code(s) through the use of a computer program which identifies features therein and identifying features within the nucleic acid code(s) or polypeptide code(s) with the computer program. In one embodiment, computer program comprises a

computer program which identifies open reading frames. In a further embodiment, the computer program comprises a computer program which identifies linear or structural motifs in a polypeptide sequence. In another embodiment, the computer program comprises a molecular modeling program. The method may be performed by reading a single sequence or at least 2, 5, 10, 15, 20, 25, 30, or 50 of the cDNA codes of SEQ ID NOS. 24-73 or the polypeptide codes of SEQ ID NOS. 74-123 through the use of the computer program and identifying features within the cDNA codes or polypeptide codes with the computer program.

The cDNA codes of SEQ ID NOS. 24-73 or the polypeptide codes of SEQ ID NOS. 74-123 may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, the cDNA codes of SEQ ID NOS. 24-73 or the polypeptide codes of SEQ ID NOS. 74-123 may be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparers, identifiers, or sources of reference nucleotide or polypeptide sequences to be compared to the cDNA codes of SEQ ID NOS. 24-73 or the polypeptide codes of SEQ ID NOS. 74-123. The following list is intended not to limit the invention but to provide guidance to programs and databases which are useful with the cDNA codes of SEQ ID NOS. 24-73 or the polypeptide codes of SEQ ID NOS. 74-123. The programs and databases which may be used include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applications Group), MacLook (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et al, *J. Mol. Biol.* 215: 403 (1990)), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 85: 2444 (1988)), FASTDB (Brutlag et al. *Comp. App. Biosci.* 6:237-245, 1990), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius<sup>2</sup>.DBAccess (Molecular Simulations Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMM (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer (Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the EMBL/Swissprotein database, the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwent's World Drug Index database, the BioByteMasterFile database, the Genbank database, and the Genseqn database. Many other programs and data bases would be apparent to one of skill in the art given the present disclosure.

Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and

beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

#### EXAMPLE 55

5

##### Methods of Making Nucleic Acids

The present invention also comprises methods of making the cDNA of SEQ ID Nos.24-73, genomic DNA obtainable therefrom, or fragment thereof. The methods comprise sequentially linking together nucleotides to produce the nucleic acids having the preceding sequences. A variety of methods of synthesizing nucleic acids are known to those skilled in the art.

10

In many of these methods, synthesis is conducted on a solid support. These included the 3' phosphoramidite methods in which the 3' terminal base of the desired oligonucleotide is immobilized on an insoluble carrier. The nucleotide base to be added is blocked at the 5' hydroxyl and activated at the 3' hydroxyl so as to cause coupling with the immobilized nucleotide base. Deblocking of the new immobilized nucleotide compound and repetition of the cycle will produce the desired polynucleotide. Alternatively, polynucleotides may be prepared as described in U.S. Patent No. 5,049,656. In some embodiments, several polynucleotides prepared as described above are ligated together to generate longer polynucleotides having a desired sequence.

15

#### EXAMPLE 56

##### Methods of Making Polypeptides

20

The present invention also comprises methods of making the polynucleotides encoded by the cDNA of SEQ ID Nos.24-73, genomic DNA obtainable therefrom, or fragments thereof and methods of making the polypeptides of SEQ ID Nos.74-123 or fragments thereof. The methods comprise sequentially linking together amino acids to produce the nucleic polypeptides having the preceding sequences. In some embodiments, the polypeptides made by these methods are 150 amino acids or less in length. In other embodiments, the polypeptides made by these methods are 120 amino acids or less in length.

25

A variety of methods of making polypeptides are known to those skilled in the art, including methods in which the carboxyl terminal amino acid is bound to polyvinyl benzene or another suitable resin. The amino acid to be added possesses blocking groups on its amino moiety and any side chain reactive groups so that only its carboxyl moiety can react. The carboxyl group is activated with carbodiimide or another activating agent and allowed to couple to the immobilized amino acid. After removal of the blocking group, the cycle is repeated to generate a polypeptide having the desired sequence. Alternatively, the methods described in U.S. Patent No. 5,049,656 may be used.

30

#### EXAMPLE 57



Immunoaffinity Chromatography

Antibodies prepared as described above are coupled to a support. Preferably, the antibodies are monoclonal antibodies, but polyclonal antibodies may also be used. The support may be any of those typically employed in immunoaffinity chromatography, including Sepharose CL-4B (Pharmacia, Piscataway, NJ), Sepharose CL-2B (Pharmacia, Piscataway, NJ), Affi-gel 10 (Biorad, Richmond, CA), or glass beads.

The antibodies may be coupled to the support using any of the coupling reagents typically used in immunoaffinity chromatography, including cyanogen bromide. After coupling the antibody to the support, the support is contacted with a sample which contains a target polypeptide whose isolation, purification or enrichment is desired. The target polypeptide may be a polypeptide of SEQ ID NOs. 74-123, a fragment thereof, or a fusion protein comprising a polypeptide of SEQ ID NOs. 74-123 or a fragment thereof.

Preferably, the sample is placed in contact with the support for a sufficient amount of time and under appropriate conditions to allow at least 50% of the target polypeptide to specifically bind to the antibody coupled to the support.

Thereafter, the support is washed with an appropriate wash solution to remove polypeptides which have non-specifically adhered to the support. The wash solution may be any of those typically employed in immunoaffinity chromatography, including PBS, Tris-lithium chloride buffer (0.1M lysine base and 0.5M lithium chloride, pH 8.0), Tris-hydrochloride buffer (0.05M Tris-hydrochloride, pH 8.0), or Tris/Triton/NaCl buffer (50mM Tris.cl, pH 8.0 or 9.0, 0.1% Triton X-100, and 0.5MNaCl).

After washing, the specifically bound target polypeptide is eluted from the support using the high pH or low pH elution solutions typically employed in immunoaffinity chromatography. In particular, the elution solutions may contain an eluant such as triethanolamine, diethylamine, calcium chloride, sodium thiocyanate, potasssium bromide, acetic acid, or glycine. In some embodiments, the elution solution may also contain a detergent such as Triton X-100 or octyl- $\beta$ -D-glucoside.

As discussed above, the cDNAs of the present invention or fragments thereof can be used for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination for expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which

binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris *et al.*, Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

5       The proteins or polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially  
10   expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen  
15   for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning; A Laboratory  
20   Manual", 2d ed., Cole Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology; Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use  
25   as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

30       Although this invention has been described in terms of certain preferred embodiments, other embodiments which will be apparent to those of ordinary skill in the art in view of the disclosure herein are also within the scope of this invention. Accordingly, the scope of the invention is intended to be defined only by reference to the appended claims. All documents cited herein are incorporated herein by reference in their entirety.

**Table I**

<b>Id</b>	<b>FCS Location</b>	<b>SigPep Location</b>	<b>Mature Polypeptide Location</b>	<b>Stop Codon Location</b>	<b>PolyA Signal Location</b>	<b>PolyA Site Location</b>
24	153/1127	153/230	231/1127	1128	1415/1420	1434/1450
25	261/1166	261/314	315/1166	1167	-	1524/1556
26	67/813	67/111	112/813	814	1023/1028	1042/1058
27	187/438	-	187/438	439	612/617	632/648
28	92/1753	92/130	131/1753	1754	2070/2075	2090/2104
29	144/440	144/287	288/440	441	457/462	500/515
30	174/443	174/269	270/443	444	623/628	647/661
31	55/399	55/192	193/399	400	654/659	680/694
32	90/287	90/146	147/287	288	1078/1083	1096/1110
33	49/447	49/111	112/447	448	579/584	602/623
34	199/618	199/408	409/618	619	626/631	643/657
35	271/969	271/366	367/969	970	1092/1097	1123/1137
36	192/440	192/278	279/440	441	590/595	622/636
37	59/703	59/181	182/703	704	783/788	804/818
38	139/1389	139/198	199/1389	1390	1854/1859	1873/1888
39	21/1118	21/89	90/1118	1119	1858/1863	1879/1894
40	143/592	143/277	278/592	593	1877/1882	1899/1913
41	76/999	76/279	280/999	1000	1711/1716	1729/1744
42	123/464	123/269	270/464	465	908/913	931/946
43	85/1230	85/129	130/1230	1231	1589/1594	1607/1622
44	29/664	29/619	620/664	665	657/662	699/715
45	18/878	18/95	96/878	879	1500/1505	1533/1549
46	73/1008	73/147	148/1008	1009	1286/1291	1312/1328
47	165/842	165/251	252/842	843	1474/1479	1500/1515
48	31/1248	31/135	136/1248	1249	1580/1585	1607/1622
49	131/490	131/301	302/490	491	1411/1416	1434/1448
50	61/690	61/168	169/690	691	858/863	879/894
51	501/1253	501/1229	1230/1253	1254	1392/1397	1432/1447
52	25/402	25/96	97/402	403	1500/1505	1525/1540
53	280/678	280/411	412/678	679	1606/1611	1628/1643
54	64/726	64/147	148/726	727	1279/1284	1300/1314
55	42/1097	42/110	111/1097	1098	2323/2328	2341/2356
56	245/1399	245/796	797/1399	1400	1669/1674	1687/1701
57	235/441	235/303	304/441	442	-	758/772
58	88/411	88/234	235/411	412	938/943	964/987
59	129/452	129/212	213/452	453	1290/1295	1309/1324
60	238/612	238/348	349/612	613	1885/1890	1905/1918
61	229/735	229/492	493/735	736	816/821	841/852

<b>Id</b>	<b>FCS Location</b>	<b>SigPep Location</b>	<b>Mature Polypeptide Location</b>	<b>Stop Codon Locatio n</b>	<b>PolyA Signal Location</b>	<b>PolyA Site Location</b>
62	168/413	168/335	336/413	414	684/689	708/726
63	100/852	100/159	160/852	853	998/1003	1019/1039
64	238/1152	238/339	340/1152	1153	1298/1303	1324/1355
65	187/369	187/312	313/369	370	489/494	558/572
66	121/459	121/165	166/459	460	497/502	521/535
67	34/336	34/123	124/336	337	536/541	556/572
68	119/409	119/388	389/409	410	769/774	789/804
69	232/534	232/306	307/534	535	595/600	615/629
70	140/595	140/442	443/595	596	630/635	655/669
71	32/658	32/289	290/658	659	936/941	959/973
72	14/280	14/76	77/280	281	-	776/791
73	93/290	93/149	150/290	291	1078/1083	1096/1110

**Table II**

<b>Seq Id No</b>	<b>Full Length Polypeptide Location</b>	<b>Signal Peptide Location</b>	<b>Mature Polypeptide Location</b>
74	-26 through 299	-26 through -1	1 through 299
75	-18 through 284	-18 through -1	1 through 284
76	-15 through 234	-15 through -1	1 through 234
77	1 through 84	-	1 through 84
78	-13 through 541	-13 through -1	1 through 541
79	-48 through 51	-48 through -1	1 through 51
80	-32 through 58	-32 through -1	1 through 58
81	-46 through 69	-46 through -1	1 through 69
82	-19 through 47	-19 through -1	1 through 47
83	-21 through 112	-21 through -1	1 through 112
84	-70 through 70	-70 through -1	1 through 70
85	-32 through 201	-32 through -1	1 through 201
86	-29 through 54	-29 through -1	1 through 54
87	-41 through 174	-41 through -1	1 through 174
88	-20 through 397	-20 through -1	1 through 397
89	-23 through 343	-23 through -1	1 through 343
90	-45 through 105	-45 through -1	1 through 105
91	-68 through 240	-68 through -1	1 through 240
92	-49 through 65	-49 through -1	1 through 65
93	-15 through 367	-15 through -1	1 through 367
94	-197 through 15	-197 through -1	1 through 15
95	-26 through 261	-26 through -1	1 through 261
96	-25 through 287	-25 through -1	1 through 287
97	-29 through 197	-29 through -1	1 through 197
98	-35 through 371	-35 through -1	1 through 371
99	-57 through 63	-57 through -1	1 through 63
100	-36 through 174	-36 through -1	1 through 174
101	-243 through 8	-243 through -1	1 through 8
102	-24 through 102	-24 through -1	1 through 102
103	-44 through 89	-44 through -1	1 through 89
104	-28 through 193	-28 through -1	1 through 193
105	-23 through 329	-23 through -1	1 through 329
106	-184 through 201	-184 through -1	1 through 201
107	-23 through 46	-23 through -1	1 through 46
108	-49 through 59	-49 through -1	1 through 59
109	-28 through 80	-28 through -1	1 through 80
110	-37 through 88	-37 through -1	1 through 88
111	-88 through 81	-88 through -1	1 through 81
112	-56 through 26	-56 through -1	1 through 26
113	-20 through 231	-20 through -1	1 through 231
114	-34 through 271	-34 through -1	1 through 271
115	-42 through 19	-42 through -1	1 through 19

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116	-15 through 98	-15 through -1	1 through 98
117	-30 through 71	-30 through -1	1 through 71
118	-90 through 7	-90 through -1	1 through 7
119	-25 through 76	-25 through -1	1 through 76
120	-101 through 51	-101 through -1	1 through 51
121	-86 through 123	-86 through -1	1 through 123
122	-21 through 68	-21 through -1	1 through 68
123	-19 through 47	-19 through -1	1 through 47

**TABLE III**

<b>Id</b>	<b>Positions of preferred fragments</b>
24	1-126, 164-259, 420-432, 1404-1450
25	32-44, 4199-1556
26	1-19, 1011-1058
27	1-16, 108-159, 595-648
28	1-119, 486-665, 1968-2009, 2055-2104
29	424-435, 500-515
30	1-122, 242-661
31	1-16, 649-694
32	1-663, 1070-110
33	1-129, 541-623
34	1-200, 614-657
35	1-419, 1094-1137
36	1-127, 323-331, 595-636
37	804-818
38	1-47, 438-611, 1005-1133, 1846-1888
39	1-430, 527-1894
40	1-119, 1743-1792, 1866-1913
41	1-70, 133-1235, 1729-1744
42	575-615, 896-946
43	513-526, 950-960, 1577-1622
44	1-2, 210-265, 674-715
45	1400-1441, 1508-1549
46	1-4, 1284, 1328

**Table IVa**

<b>Seq Id N°</b>	<b>Preferred fragments</b>
24	1-58:343-1359:1434-1450
25	455-1556
26	553-634:1042-1058
27	608-648
28	452-481:620-2104
29	424-515
30	497-661
31	529-694
32	639-1110
33	505-623
34	536-657
35	444-1137
36	593-636
37	448-818
38	643-1346:1809-1888
39	276-1894
40	332-1913
41	392-1744
42	578-946
43	1-240:645-1224:1341-1622
44	695-715
45	472-706:924-1549
46	495-1328
47	440-1193:1494-1515
48	532-1024:1065-1622
49	495-582:1412-1448
50	427-894
51	500-1321:1424-1447
52	487-1540
53	441-1272:1330-1643
54	915-1314
55	453-2356
56	519-1701
57	550-772
58	340-987
59	467-1324
60	442-1918
61	521-852
62	452-726
63	128-143:481-1039
64	492-1355
65	527-572
66	521-535
67	526-572
68	512-804
69	552-629
70	655-669
71	423-973



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72	529-791
73	642-1110

**Table IVb**

<b>Seq Id N°</b>	<b>Excluded fragments</b>
24	59-342:1360-1433
25	1-454
26	1-552:635-1041
27	1-607
28	1-451:482-619
29	1-423
30	1-496
31	1-528
32	1-638
33	1-504
34	1-535
35	1-443
36	1-592
37	1-447
38	1-642:1347-1808
39	1-275
40	1-331
41	1-391
42	1-577
43	241-644:1225-1340
44	1-694
45	1-471:707-923
46	1-494
47	1-439:1194-1493
48	1-531:1025-1064
49	1-494:583-1411
50	1-426
51	1-499:1322-1423
52	1-486
53	1-440:1273-1329
54	1-914
55	1-452
56	1-518
57	1-549
58	1-339
59	1-466
60	1-441
61	1-520
62	1-451
63	1-127:144-480
64	1-491
65	1-526
66	1-520
67	1-525
68	1-511
69	1-551
70	1-654
71	1-422

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72	1-528
73	1-641

**Table V**

<b>Internal designation</b>	<b>Id</b>	<b>Type of sequence</b>
105-016-3-0-E3-FL	24	DNA
105-031-3-0-D6-FL	25	DNA
105-095-1-0-D10-FL	26	DNA
105-118-4-0-E6-FL	27	DNA
114-025-2-0-F11-FL	28	DNA
116-005-4-0-G11-FL	29	DNA
116-032-2-0-F9-FL	30	DNA
116-047-3-0-B1-FL	31	DNA
116-048-4-0-A6-FL	32	DNA
116-049-1-0-F2-FL	33	DNA
116-050-2-0-A11-FL	34	DNA
116-054-3-0-E6-FL	35	DNA
116-054-3-0-G12-FL	36	DNA
116-073-4-0-C8-FL	37	DNA
117-002-3-0-G3-FL	38	DNA
117-005-2-0-E10-FL	39	DNA
117-005-3-0-F2-FL	40	DNA
117-005-4-0-E5-FL	41	DNA
117-007-2-0-B5-FL	42	DNA
117-007-2-0-C4-FL	43	DNA
121-004-3-0-F6-FL	44	DNA
122-005-2-0-F11-FL	45	DNA
122-007-3-0-D10-FL	46	DNA
108-004-5-0-B12-FL	47	DNA
108-004-5-0-C10-FL	48	DNA
108-004-5-0-G10-FL	49	DNA
108-005-5-0-D4-FL	50	DNA
108-005-5-0-F9-FL	51	DNA
108-006-5-0-C7-FL	52	DNA
108-006-5-0-E1-FL	53	DNA
108-008-5-0-C5-FL	54	DNA
108-008-5-0-G5-FL	55	DNA
108-011-5-0-B12-FL	56	DNA
108-011-5-0-C7-FL	57	DNA
108-011-5-0-G8-FL	58	DNA
108-011-5-0-H2-FL	59	DNA
108-013-5-0-G5-FL	60	DNA
108-013-5-0-H9-FL	61	DNA
108-014-5-0-A10-FL	62	DNA
108-014-5-0-C7-FL	63	DNA
108-014-5-0-D12-FL	64	DNA
108-014-5-0-H8-FL	65	DNA
108-015-5-0-E2-FL	66	DNA
108-016-5-0-C12-FL	67	DNA
108-016-5-0-D4-FL	68	DNA
108-019-5-0-F10-FL	69	DNA
108-019-5-0-F5-FL	70	DNA
108-019-5-0-H3-FL	71	DNA
108-020-5-0-D4-FL	72	DNA

Internal designation	Id	Type of sequence
108-020-5-0-E3-FL	73	DNA
105-016-3-0-E3-FL	74	PRT
105-031-3-0-D6-FL	75	PRT
105-095-1-0-D10-FL	76	PRT
105-118-4-0-E6-FL	77	PRT
114-025-2-0-F11-FL	78	PRT
116-005-4-0-G11-FL	79	PRT
116-032-2-0-F9-FL	80	PRT
116-047-3-0-B1-FL	81	PRT
116-048-4-0-A6-FL	82	PRT
116-049-1-0-F2-FL	83	PRT
116-050-2-0-A11-FL	84	PRT
116-054-3-0-E6-FL	85	PRT
116-054-3-0-G12-FL	86	PRT
116-073-4-0-C8-FL	87	PRT
117-002-3-0-G3-FL	88	PRT
117-005-2-0-E10-FL	89	PRT
117-005-3-0-F2-FL	90	PRT
117-005-4-0-E5-FL	91	PRT
117-007-2-0-B5-FL	92	PRT
117-007-2-0-C4-FL	93	PRT
121-004-3-0-F6-FL	94	PRT
122-005-2-0-F11-FL	95	PRT
122-007-3-0-D10-FL	96	PRT
108-004-5-0-B12-FL	97	PRT
108-004-5-0-C10-FL	98	PRT
108-004-5-0-G10-FL	99	PRT
108-005-5-0-D4-FL	100	PRT
108-005-5-0-F9-FL	101	PRT
108-006-5-0-C7-FL	102	PRT
108-006-5-0-E1-FL	103	PRT
108-008-5-0-C5-FL	104	PRT
108-008-5-0-G5-FL	105	PRT
108-011-5-0-B12-FL	106	PRT
108-011-5-0-C7-FL	107	PRT
108-011-5-0-G8-FL	108	PRT
108-011-5-0-H2-FL	109	PRT
108-013-5-0-G5-FL	110	PRT
108-013-5-0-H9-FL	111	PRT
108-014-5-0-A10-FL	112	PRT
108-014-5-0-C7-FL	113	PRT
108-014-5-0-D12-FL	114	PRT
108-014-5-0-H8-FL	115	PRT
108-015-5-0-E2-FL	116	PRT
108-016-5-0-C12-FL	117	PRT
108-016-5-0-D4-FL	118	PRT
108-019-5-0-F10-FL	119	PRT
108-019-5-0-F5-FL	120	PRT
108-019-5-0-H3-FL	121	PRT
108-020-5-0-D4-FL	122	PRT
108-020-5-0-E3-FL	123	PRT

**Table VI**

Seq Id No	Tissue expression
24	prostate:2
25	fetal kidney:1 prostate:3
27	prostate:1
28	liver:1
29	testis:1
30	testis:3
31	testis:1
32	testis:1
33	testis:1
34	liver:1 testis:3
35	liver:1 testis:3
36	testis:1
37	testis:1
38	liver:2
39	liver:3
40	liver:1
41	liver:1
42	brain:2 liver:1 placenta:6 salivary gland:1
44	fetal brain:6
45	fetal brain:6 placenta:2
46	fetal brain:9
47	prostate:2
48	prostate:3
49	prostate:1
50	prostate:1
51	prostate:3
52	prostate:3
53	prostate:2
54	prostate:1
55	prostate:1
56	liver:15 testis:3
57	liver:1 testis:8
58	brain:1
59	prostate:1
60	liver:15
61	prostate:2
62	testis:1
63	testis:3
64	liver:2
65	liver:1 testis:2
66	liver:5 testis:20

67	brain:4 fetal brain:10 fetal kidney:1 fetal livery:1 placenta:1 prostate:1
68	brain:3 fetal brain:4 fetal kidney:7 prostate:1 salivary gland:1 testis:2
69	liver:1 testis:1
70	fetal livery:1 prostate:1 salivary gland:3 stomach/intestine:2 testis:1
71	testis:1
72	fetal brain:4
73	brain:85

**Table VII**

<b>Seq Id No</b>	<b>Preferential expression</b>
24	Prostate
25	Prostate
27	Prostate
28	None
29	None
30	Testis
31	None
32	None
33	None
34	Testis
35	Testis
36	None
37	None
38	Liver
39	Liver
40	None
41	None
42	Placenta
44	Fetal brain
45	None
46	Fetal brain
47	Prostate
48	Prostate
49	Prostate
50	Prostate
51	Prostate
52	Prostate
53	Prostate
54	Prostate
55	Prostate
56	Liver
57	Testis
58	None
59	Prostate
60	Liver
61	Prostate
62	None
63	Testis
64	Liver
65	None
66	Testis



67	None
68	Fetal kidney
69	None
70	Salivary gland, Stomach/Intestine
71	None
72	Fetal brain
73	Brain

**Table VIII**

Seq Id No	Public expression
24	frontal lobe(2)
25	B-cell, chronic lymphocytic leukemia(2), "adenocarcinoma"(2), "germinal center B cell"(2), "liver"(1), "lung"(1), "tumor"(1)
27	2 pooled tumors (clear cell type)(5), "adenocarcinoma"(1), "anaplastic oligodendroglioma"(4), "brain"(3), "breast"(4), "breast tumor"(1), "carcinoid"(5), "cerebellum"(1), "colon"(4), "colon tumor RER+"(2), "frontal lobe"(5), "germinal center B cell"(4), "glioblastoma (pooled)"(2), "moderately-differentiated adenocarcinoma"(1), "normal prostate"(3), "ovary"(2), "parathyroid tumor"(4), "pectoral muscle (after mastectomy)"(1), "pooled germ cell tumors"(5), "senescent fibroblast"(4), "tumor"(1), "tumor, 5 pooled (see description)"(1)
28	colon(1), "neuroepithelial cells"(1)
29	2 pooled tumors (clear cell type)(2), "anaplastic oligodendroglioma"(2), "borderline ovarian carcinoma"(1), "carcinoid"(3), "colon"(1), "epithelium (cell line)"(1), "glioblastoma (pooled)"(1), "ovarian tumor"(1), "pooled germ cell tumors"(2)
30	NONE
31	2 pooled tumors (clear cell type)(5), "breast"(1), "carcinoid"(1), "colon tumor, RER+"(1), "kidney tumor"(1), "pooled germ cell tumors"(1)
32	NONE
33	2 pooled tumors (clear cell type)(2)
34	NONE
35	NONE
36	2 pooled tumors (clear cell type)(4), "breast"(1), "prostate"(1)
37	pooled germ cell tumors(1)
38	NONE
39	liver(2)
40	B-cell, chronic lymphocytic leukemia(2), "brain"(1), "carcinoid"(1), "colon"(1)
41	NONE
42	anaplastic oligodendroglioma(2), "cerebellum"(1), "colon"(1), "glioblastoma (pooled)"(5), "metastatic prostate bone lesion"(1), "normal epithelium"(1), "parathyroid tumor"(1), "pooled germ cell tumors"(1), "renal cell tumor"(1), "retina"(2), "squamous cell carcinoma"(1), "squamous cell carcinoma from base of tongue"(1), "three pooled meningiomas"(1)
44	anaplastic oligodendroglioma(1), "brain"(1), "frontal lobe"(6), "total brain"(2)
45	Lung(1), "muscle"(1), "parathyroid tumor"(1), "synovial membrane"(1)
46	neuroepithelial cells(1), "total brain"(1)
47	Bone(1), "bone marrow stroma"(1), "brain"(1), "testis"(1)
48	NONE
49	parathyroid tumor(1), "retina"(1), "total brain"(2)
50	NONE
51	ovarian tumor(3), "retina"(1), "senescent fibroblast"(1)

52	normal prostate(1)
53	NONE
54	foreskin(1)
55	NONE
56	NONE
57	NONE
58	NONE
59	adenocarcinoma(1), "pectoral muscle (after mastectomy)"(1)
60	juvenile granulosa tumor(1), "liver"(1), "senescent fibroblast"(1)
61	2 pooled tumors (clear cell type)(2), "germinal center B cell"(6)
62	NONE
63	NONE
64	NONE
65	NONE
66	NONE
67	B-cell, chronic lymphocytic leukemia(1), "adenocarcinoma"(1), "anaplastic oligodendroglioma"(3), "carcinoid"(3), "frontal lobe"(2), "glioblastoma (pooled)"(4), "normal epithelium"(1), "pooled germ cell tumors"(1)
68	2 pooled tumors (clear cell type)(5), "Lung"(1), "adenocarcinoma"(4), "adipose tissue, white"(1), "adrenal adenoma"(1), "anaplastic oligodendroglioma"(2), "breast tumor"(1), "carcinoid"(1), "colon"(4), "epithelium (cell line)"(1), "liver"(1), "melanocyte"(1), "ovarian tumor"(1), "parathyroid tumor"(6), "pectoral muscle (after mastectomy)"(4), "squamous cell carcinoma"(1), "synovial membrane"(3)
69	NONE
70	2 pooled tumors (clear cell type)(1), "anaplastic oligodendroglioma"(2), "carcinoid"(3), "colon"(4), "epithelium (cell line)"(1), "glioblastoma (pooled)"(1), "normal prostate"(2), "ovarian tumor"(2), "pooled germ cell tumors"(3), "senescent fibroblast"(2), "testis"(1)
71	NONE
72	anaplastic oligodendroglioma(2), "astrocytoma"(1), "glioblastoma (pooled)"(1), "total brain"(1)
73	NONE

**Table IX**

Seq Id No	Positions	Motif designation	Database
74	none	none	none
75	none	none	none
76	none	none	none
77	33-79	PHD	Pfam
78	none	none	none
79	none	none	none
80	none	none	none
81	28-94	pfkB	Pfam
82	none	none	none
83	none	none	none
84	none	none	none
85	none	none	none
86	none	none	none
87	88-213	lys	Pfam
87	183-202	BL00128C Alpha-lactalbumin / lysozyme C signature	BLOCKSPLUS
87	111-120	PR00135B LYSOZYME/ALPHA-LACTALBUMIN SUPERFAMILY SIGNATURE	BLOCKSPLUS
87	162-180	Alpha-lactalbumin / lysozyme C signature	PROSITE
88	246-266	PSAP	Pfam
89	92-207	NusB	Pfam
89	4-251	Apolipoprotein	Pfam
89	110-263	Nop	Pfam
90	none	none	none
91	2-134	mito_carr 1/2	Pfam
91	156-303	mito_carr 2/2	Pfam
91	5-29	BL00215A Mitochondrial energy transfer proteins	BLOCKSPLUS
91	223-247	BL00215A Mitochondrial energy transfer proteins	BLOCKSPLUS
91	102-125	BL00215A Mitochondrial energy transfer proteins	BLOCKSPLUS
91	169-182	BL00215B Mitochondrial energy transfer proteins	BLOCKSPLUS
92	none	none	none
93	37-104	cystatin 1/2	Pfam
93	157-254	cystatin 2/2	Pfam
94	105-154	GST	Pfam
95	27-131	Cyt_reductase	Pfam
95	158-272	oxidored_fad	Pfam
95	256-265	PR00406F CYTOCHROME B5 REDUCTASE SIGNATURE	BLOCKSPLUS
95	123-138	PR00406C CYTOCHROME B5 REDUCTASE SIGNATURE	BLOCKSPLUS
95	256-268	BL00559L Eukaryotic molybdopterin oxidoreductases proteins	BLOCKSPLUS

95	163-180	PR00406D CYTOCHROME B5 REDUCTASE SIGNATURE	BLOCKSPLUS
95	163-179	PR00371D FLAVOPROTEIN PYRIDINE NUCLEOTIDE CYTOCHROME REDUCTASE SIGNATURE	BLOCKSPLUS
95	110-120	PR00371C FLAVOPROTEIN PYRIDINE NUCLEOTIDE CYTOCHROME REDUCTASE SIGNATURE	BLOCKSPLUS
96	7-27	PR00953B FLAGELLAR BIOSYNTHETIC PROTEIN FLIR SIGNATURE	BLOCKSPLUS
97	none	none	none
98	none	none	none
99	none	none	none
100	none	none	none
101	7-214	Hydrolase	Pfam
102	48-53	Cytochrome c family heme-binding site	PROSITE
102	24-26	Protein kinase C phosphorylation site	PROSITE
103	none	none	none
104	none	none	none
105	302-339	zf-C3HC4	Pfam
106	none	none	none
107	17-67	maseA	Pfam
108	none	none	none
109	none	none	none
110	17-40	A2M_N	Pfam
111	52-66	PR00111B ALPHA/BETA HYDROLASE FOLD SIGNATURE	BLOCKSPLUS
112	none	none	none
113	59-61	Cell attachment sequence	PROSITE
114	258-298	zf-C3HC4	Pfam
114	257-301	PHD	Pfam
115	none	none	none
116	none	none	none
117	none	none	none
118	none	none	none
119	none	none	none
120	none	none	none
121	none	none	none
122	none	none	none
123	none	none	none

**Table X**

Seq Id No	Antigenic epitopes
74	58, 86-88, 148-149, 175-177, 238-239, 319
75	43-45, 58, 63-64, 72-74, 202, 204-205, 207, 237 -238, 298
76	119, 121
77	21, 40-43
78	41,43-44, 83, 103-104, 184-185, 187-188, 210-212, 366-367, 372-373, 396-397, 421, 475-477
79	84, 86-87
80	17, 37-38, 40-41, 43-44
81	97-98
82	34
83	20, 26-30, 83-86, 103, 111-112, 131
84	9-10, 96-97
85	220-222, 230-231
86	36, 44-47, 50-51, 67-68, 81-83
87	44-45, 105-106, 108-109, 147-149, 173, 202-203
88	129-130, 178, 311-312, 333-335, 368-369
89	34, 36-37, 319-320, 331-333
90	60
91	31-32, 157-158, 180, 215-216, 250
92	60-61
93	35, 37-38, 54-55, 57-58, 75-76, 160-161, 183-184, 215-216, 230, 291-292, 296, 302, 309
94	5, 9, 11, 99, 184
95	61-62, 87-88, 109-110, 147-148, 216-217, 229-231, 252,273
96	83, 89, 249-250
97	34-35, 209-211
98	104-106, 199-200, 228-229, 245-246, 292, 326-327, 342-343
99	25-28, 105-106, 108-109
100	59-60, 97-98, 101-102, 106-107, 159-160, 193-194, 207-208
101	61
102	56-57, 61-63, 83-84
103	47-48, 77-80, 100, 107
104	92-93
105	3-5, 59, 112-113, 213-214
106	31-32, 66, 108-109, 148-149, 165-167, 170-172, 290-291,339-340
107	32-34, 37-38, 57
108	6-7, 9, 11-12, 56-57
109	47-49, 91-92

110	38-39, 74, 92-93, 108-109, 116
111	17, 96
112	41-43
113	34-34, 84-85
114	83-84, 135-136, 264-265
115	19-23, 41
116	44-44, 109-109
117	4-5, 7-8, 55-56, 94-95
118	31-32, 38-40, 59-60
119	54-55, 59
120	137-137, 139-140
121	56, 86
122	4-5, 58-58, 67-68, 70-72, 74-77, 82-83
123	34

**Table XI**

<b>Seq Id No</b>	<b>Chromosomal location</b>
24	none
25	9
26	20
27	17
28	8
29	16
30	1
31	none
32	none
33	none
34	none
35	none
36	none
37	17
38	12q
39	11
40	18
41	14
42	6p23-25.1
43	none
44	20q12
45	none
46	3
47	none
48	1
49	20
50	none
51	9
52	11q24
53	17
54	none
55	1
56	3
57	14
58	16
59	11
60	10
61	none
62	none
63	19
64	none
65	6
66	X
67	6p12.3-21.2



68	5
69	none
70	16
71	9
72	20
73	none

**FREE TEXT**

Von Heijne matrix  
Score  
oligonucleotide used as a primer  
5 matinspector prediction  
name  
complement

WHAT IS CLAIMED IS:

1. A purified or isolated nucleic acid comprising the sequence of one of SEQ ID NOs: 24-73 or a sequence complementary thereto.
2. A purified or isolated nucleic acid comprising at least 12 consecutive bases of the sequence of one of SEQ ID NOs: 24-73 or one of the sequences complementary thereto.
3. A purified or isolated nucleic acid comprising the full coding sequences of one of SEQ ID NOs: 24-73, wherein the full coding sequence comprises the sequence encoding signal peptide and the sequence encoding mature protein.
4. A purified or isolated nucleic acid comprising the nucleotides of one of SEQ ID NOs: 24-73 which encode a mature protein.
5. A purified or isolated nucleic acid comprising the nucleotides of one of SEQ ID NOs: 24-73 which encode the signal peptide.
6. A purified or isolated nucleic acid encoding a polypeptide having the sequence of one of the sequences of SEQ ID NOs: 74-123.
7. A purified or isolated nucleic acid encoding a polypeptide having the sequence of a mature protein included in one of the sequences of SEQ ID NOs: 74-123.
8. A purified or isolated nucleic acid encoding a polypeptide having the sequence of a signal peptide included in one of the sequences of SEQ ID NOs: 74-123.
9. A purified or isolated protein comprising the sequence of one of SEQ ID NOs: 74-123.
10. A purified or isolated polypeptide comprising at least 10 consecutive amino acids of one of the sequences of SEQ ID NOs: 74-123.
11. An isolated or purified polypeptide comprising a signal peptide of one of the polypeptides of SEQ ID NOs: 74-123.
12. An isolated or purified polypeptide comprising a mature protein of one of the polypeptides of SEQ ID NOs: 74-123.
13. A method of making a protein comprising one of the sequences of SEQ ID NO: 74-123, comprising the steps of:
  - obtaining a cDNA comprising one of the sequences of sequence of SEQ ID NO: 24-73;
  - inserting said cDNA in an expression vector such that said cDNA is operably linked to a promoter; and

introducing said expression vector into a host cell whereby said host cell produces the protein encoded by said cDNA.

14. The method of Claim 13, further comprising the step of isolating said protein.
15. A protein obtainable by the method of Claim 14.
- 5 16. A host cell containing a recombinant nucleic acid of Claim 1.
17. A purified or isolated antibody capable of specifically binding to a protein having the sequence of one of SEQ ID NOs: 74-123.
18. In an array of polynucleotides of at least 15 nucleotides in length, the improvement comprising inclusion in said array of at least one of the sequences of SEQ ID NOs: 24-73, or one of the  
10 sequences complementary to the sequences of SEQ ID NOs: 24-73, or a fragment thereof of at least 15 consecutive nucleotides.
19. A purified or isolated nucleic acid of at least 15 bases capable of hybridizing under stringent conditions to the sequence of one of SEQ ID NOs: 24-73 or a sequence complementary to one of the sequences of SEQ ID NOs: 24-73.
- 15 20. A purified or isolated antibody capable of binding to a polypeptide comprising at least 10 consecutive amino acids of the sequence of one of SEQ ID NOs: 74-123.
21. A computer readable medium having stored thereon a sequence selected from the group consisting of a cDNA code of SEQID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123.
- 20 22. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a sequence selected from the group consisting of a cDNA code of SEQID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123.
23. The computer system of Claim 22 further comprising a sequence comparer and a data storage device having reference sequences stored thereon.
- 25 24. The computer system of Claim 23 wherein said sequence comparer comprises a computer program which indicates polymorphisms.
25. The computer system of Claim 22 further comprising an identifier which identifies features in said sequence.

26. A method for comparing a first sequence to a reference sequence wherein said first sequence is selected from the group consisting of a cDNA code of SEQ ID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123 comprising the steps of:
- 5           reading said first sequence and said reference sequence through use of a computer program which compares sequences; and
- determining differences between said first sequence and said reference sequence with said computer program.
27. The method of Claim 26, wherein said step of determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.
- 10 28. A method for identifying a feature in a sequence selected from the group consisting of a cDNA code of SEQ ID NOs. 24-73 and a polypeptide code of SEQ ID NOs. 74-123 comprising the steps of:
- reading said sequence through the use of a computer program which identifies features in sequences; and
- 15           identifying features in said sequence with said computer program.
29. A purified or isolated nucleic acid comprising a contiguous span of at least 12 nucleotides of the sequence of one of SEQ ID NOs: 24-73 or one of the sequences complementary thereto, wherein said contiguous span comprises at least 1 of the nucleotide positions of polynucleotides described in Table III.
- 20 30. A purified or isolated nucleic acid comprising a contiguous span of at least 12 nucleotides of the sequence of one of the polynucleotides described in Table III or one of the sequences complementary thereto.

Step	Search characteristic		Selection Characteristics		
	Program	Strand	Parameters	Identity (%)	Length (bp)
miscellaneous	FASTA	both	-	90	15
tRNA	FASTA	both	-	80	60
rRNA	BLASTN	both	S=108	80	40
mtRNA	BLASTN	both	S=108	80	40
Prokaryotic	BLASTN	both	S=144	90	40
Fungal	BLASTN	both	S=144	90	40
Alu	BLASTN	both	S=72, B=5	70	40
L1	BLASTN	both	S=72, B=5	70	40
Repeats	BLASTN	both	S=72	70	40
PolyA	BLAST2N	top	W=6, S=10, E=1000, N=-12	90	10
Polyadenylation signal	-	top	AATAAA allowing 1 mismatch		in the 50 nucleotides preceding the 5' end of the polyA
Vertebrate	BLASTN then FASTA	both	-	90 then 70	30
ESTs	BLAST2N	both	-	90	30
Geneseq	BLASTN	both	W=8, B=10	90	30
ORF	BLASTP	top	W=8, B=10	-	on ORF proteins, max 10 matches
Proteins	BLASTX	top	E = 0.001	70	30

Parameters used for each step of cDNA analysis

Figure 1

Minimum signal peptide score	false positive rate	false negative rate	proba(0.1)	proba(0.2)
3,5	0,121	0,036	0,467	0,664
4	0,096	0,06	0,519	0,708
4,5	0,078	0,079	0,565	0,745
5	0,062	0,098	0,615	0,782
5,5	0,05	0,127	0,659	0,813
6	0,04	0,163	0,694	0,836
6,5	0,033	0,202	0,725	0,855
7	0,025	0,248	0,763	0,878
7,5	0,021	0,304	0,78	0,889
8	0,015	0,368	0,816	0,909
8,5	0,012	0,418	0,836	0,92
9	0,009	0,512	0,856	0,93
9,5	0,007	0,581	0,863	0,934
10	0,006	0,679	0,835	0,919

Figure 2

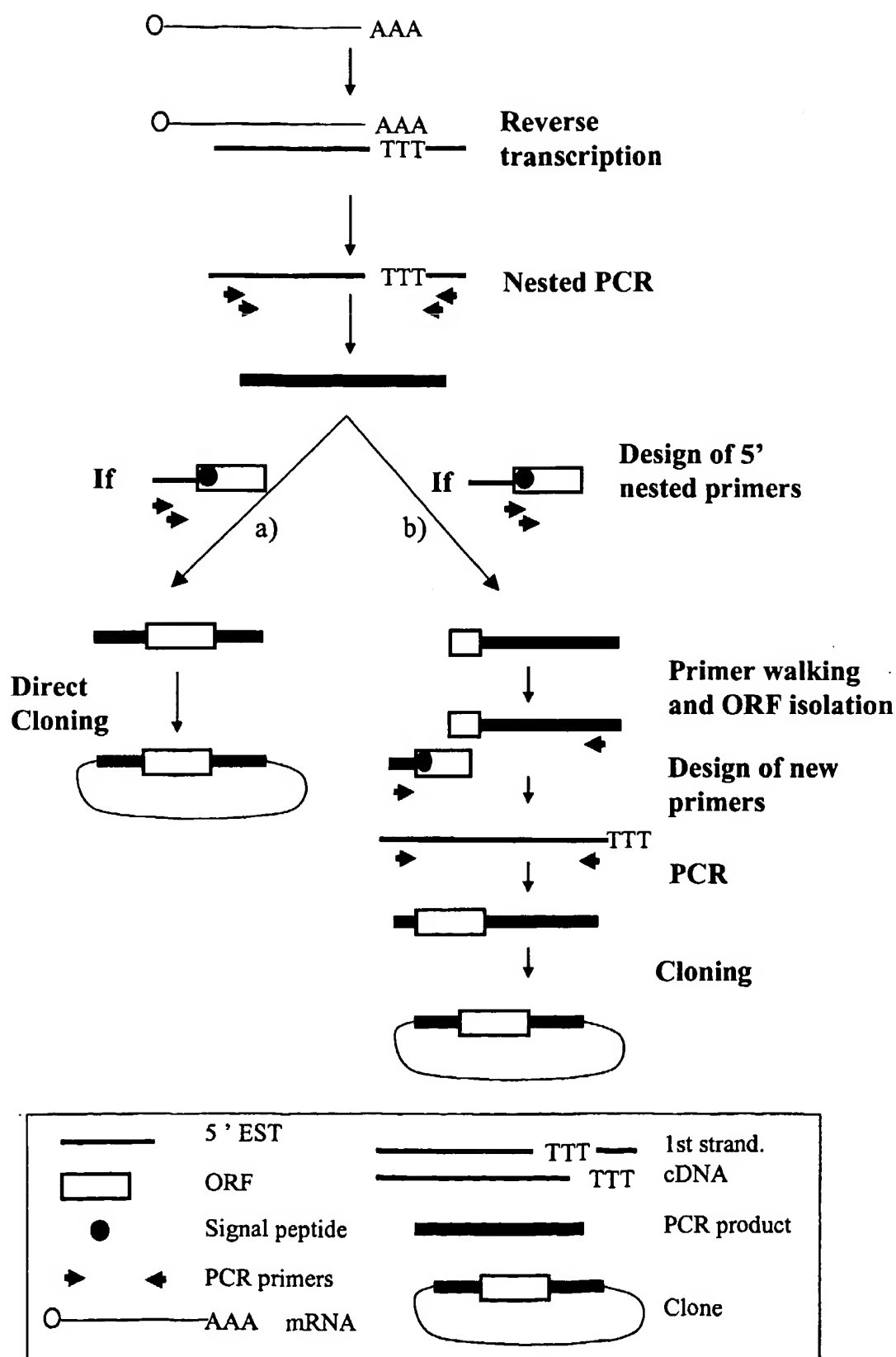


Figure 3



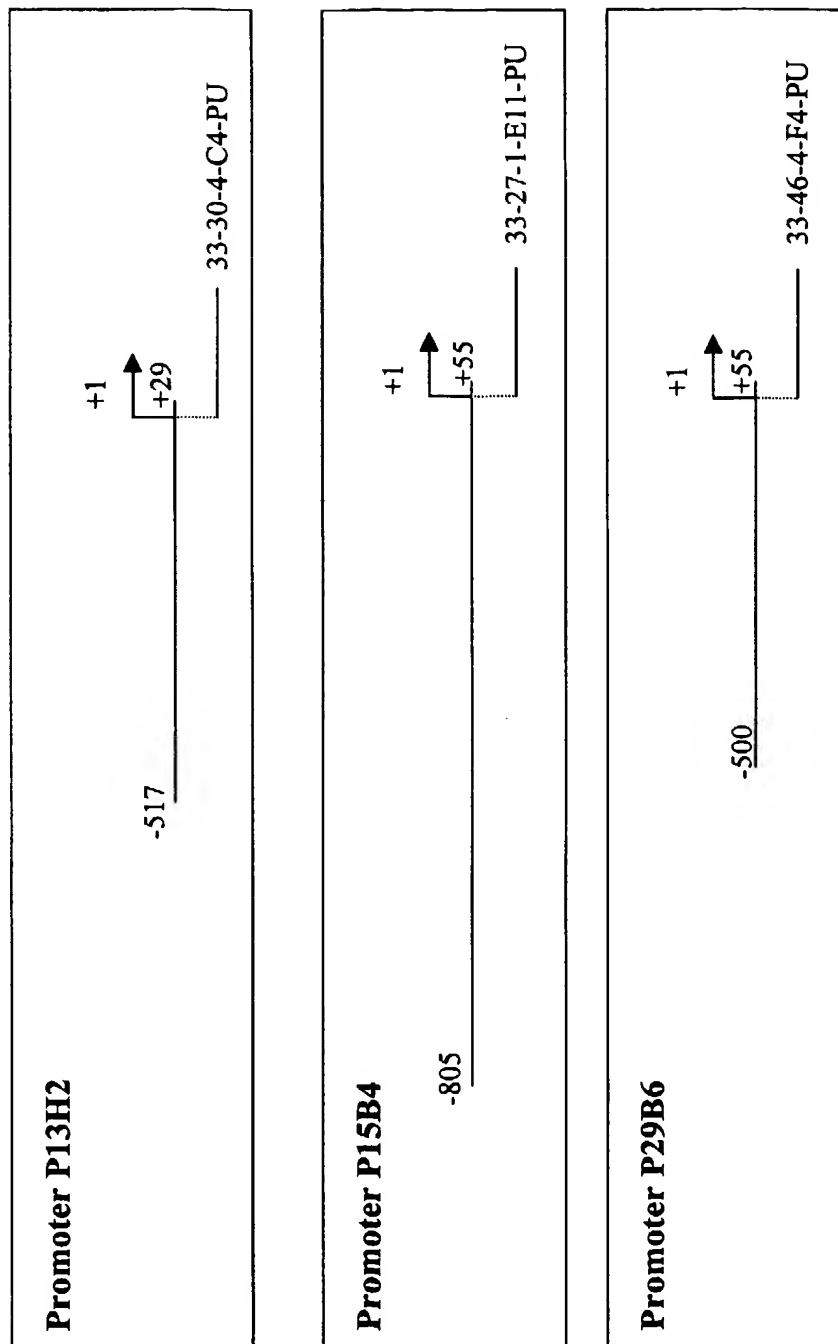


Figure 4

### Description of Transcription Factor Binding Sites present on promoters isolated from SignalTag sequences

#### Promoter sequence P13H2 (546 bp):

Matrix	Position	Orientation	Score	Length	Sequence
CMYB_01	-502	+	0.983	9	TGTCAGTTG
MYOD_Q6	-501	-	0.961	10	CCCAACTGAC
S8_01	-444	-	0.960	11	AATAGAATTAG
S8_01	-425	+	0.966	11	AACTAAATTAG
DELTAEF1_01	-390	-	0.960	11	GCACACCTCAG
GATA_C	-364	-	0.964	11	AGATAAATCCA
CMYB_01	-349	+	0.958	9	CTTCAGTTG
GATA1_02	-343	+	0.959	14	TTGTAGATAGGACA
GATA_C	-339	+	0.953	11	AGATAGGACAT
TAL1ALPHAE47_01	-235	+	0.973	16	CATAACAGATGGTAAG
TAL1BETAE47_01	-235	+	0.983	16	CATAACAGATGGTAAG
TAL1BETAIF2_01	-235	+	0.978	16	CATAACAGATGGTAAG
MYOD_Q6	-232	-	0.954	10	ACCATCTGTT
GATA1_04	-217	-	0.953	13	TCAAGATAAAGTA
IK1_01	-126	+	0.963	13	AGTTGGGAATTCC
IK2_01	-126	+	0.985	12	AGTTGGGAATTC
CREL_01	-123	+	0.962	10	TGGGAATTCC
GATA1_02	-96	+	0.950	14	TCAGTGATATGGCA
SRY_02	-41	-	0.951	12	TAAACAAAACA
E2F_02	-33	+	0.957	8	TTTAGCGC
MZF1_01	-5	-	0.975	8	TGAGGGGA

#### Promoter sequence P15B4 (861bp) :

Matrix	Position	Orientation	Score	Length	Sequence
NFY_Q6	-748	-	0.956	11	GGACCAATCAT
MZF1_01	-738	+	0.962	8	CCTGGGGA
CMYB_01	-684	+	0.994	9	TGACCGTTG
VMYB_02	-682	-	0.985	9	TCCAACGGT
STAT_01	-673	+	0.968	9	TTCTGGAA
STAT_01	-673	-	0.951	9	TTCCAGGAA
MZF1_01	-556	-	0.956	8	TTGGGGGA
IK2_01	-451	+	0.965	12	GAATGGGATTC
MZF1_01	-424	+	0.986	8	AGAGGGGA
SRY_02	-398	-	0.955	12	GAAAACAAAACA
MZF1_01	-216	+	0.960	8	GAAGGGGA
MYOD_Q6	-190	+	0.981	10	AGCATCTGCC
DELTAEF1_01	-176	+	0.958	11	TCCCACCTTCC
S8_01	5	-	0.992	11	GAGGCAATTAT
MZF1_01	16	-	0.986	8	AGAGGGGA

#### Promoter sequence P29B6 (555 bp) :

Matrix	Position	Orientation	Score	Length	Sequence
ARNT_01	-311	+	0.964	16	GGACTCACGTGCTGCT
NMYC_01	-309	+	0.965	12	ACTCACGTGCTG
USF_01	-309	+	0.985	12	ACTCACGTGCTG
USF_01	-309	-	0.985	12	CAGCACGTGAGT
NMYC_01	-309	-	0.956	12	CAGCACGTGAGT
MYCMAX_02	-309	-	0.972	12	CAGCACGTGAGT
USF_C	-307	+	0.997	8	TCACGTGC
USF_C	-307	-	0.991	8	GCACGTGA
MZF1_01	-292	-	0.968	8	CATGGGGA
ELK1_02	-105	+	0.963	14	CTCTCCGGAAGCCT
CETS1P54_01	-102	+	0.974	10	TCCGGAAGCC
AP1_Q4	-42	-	0.963	11	AGTGAAGTAAC
AP1FJ_Q2	-42	-	0.961	11	AGTGAAGTAAC
PADS_C	45	+	1.000	9	TGTGGTCTC

Figure 5

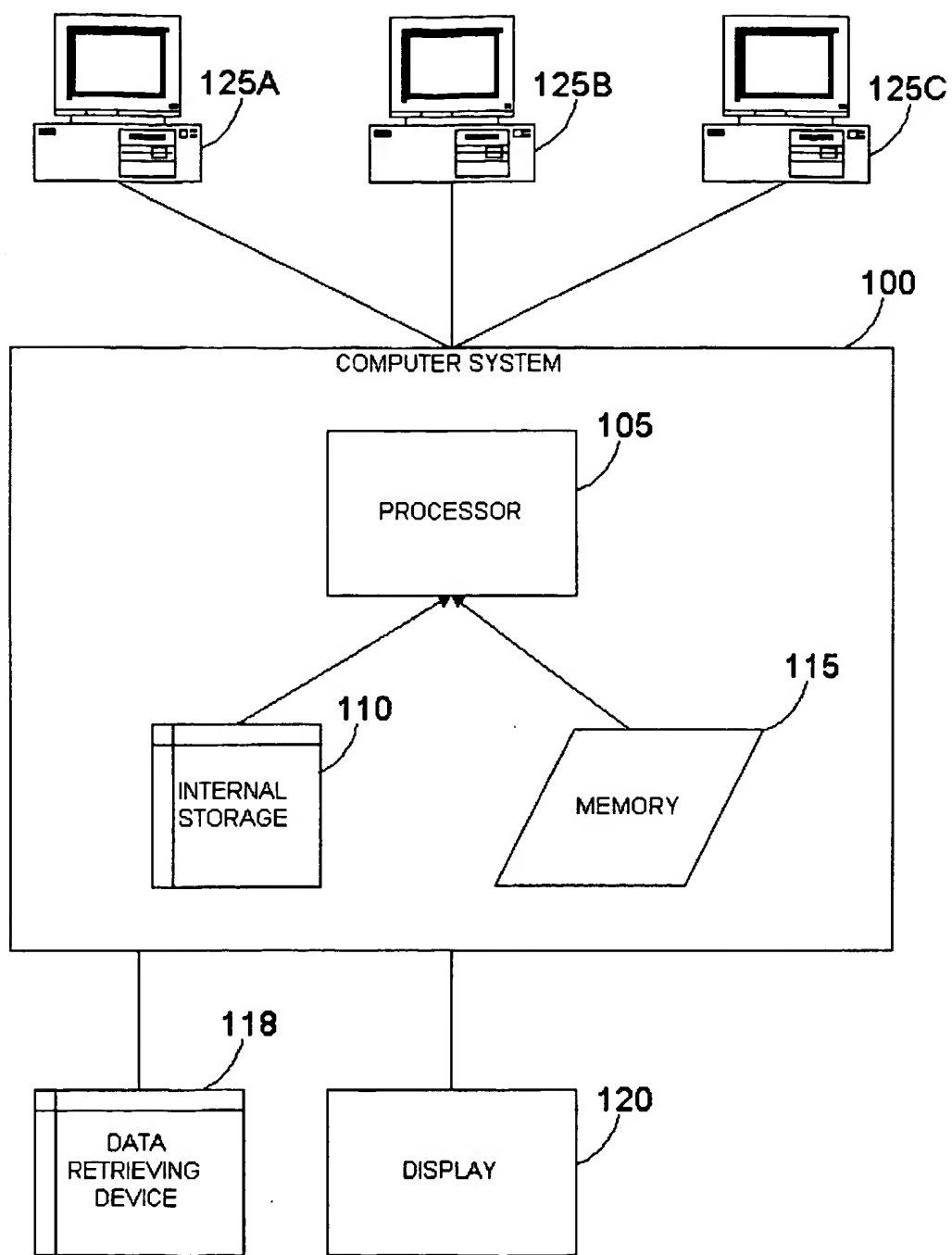


FIGURE 6

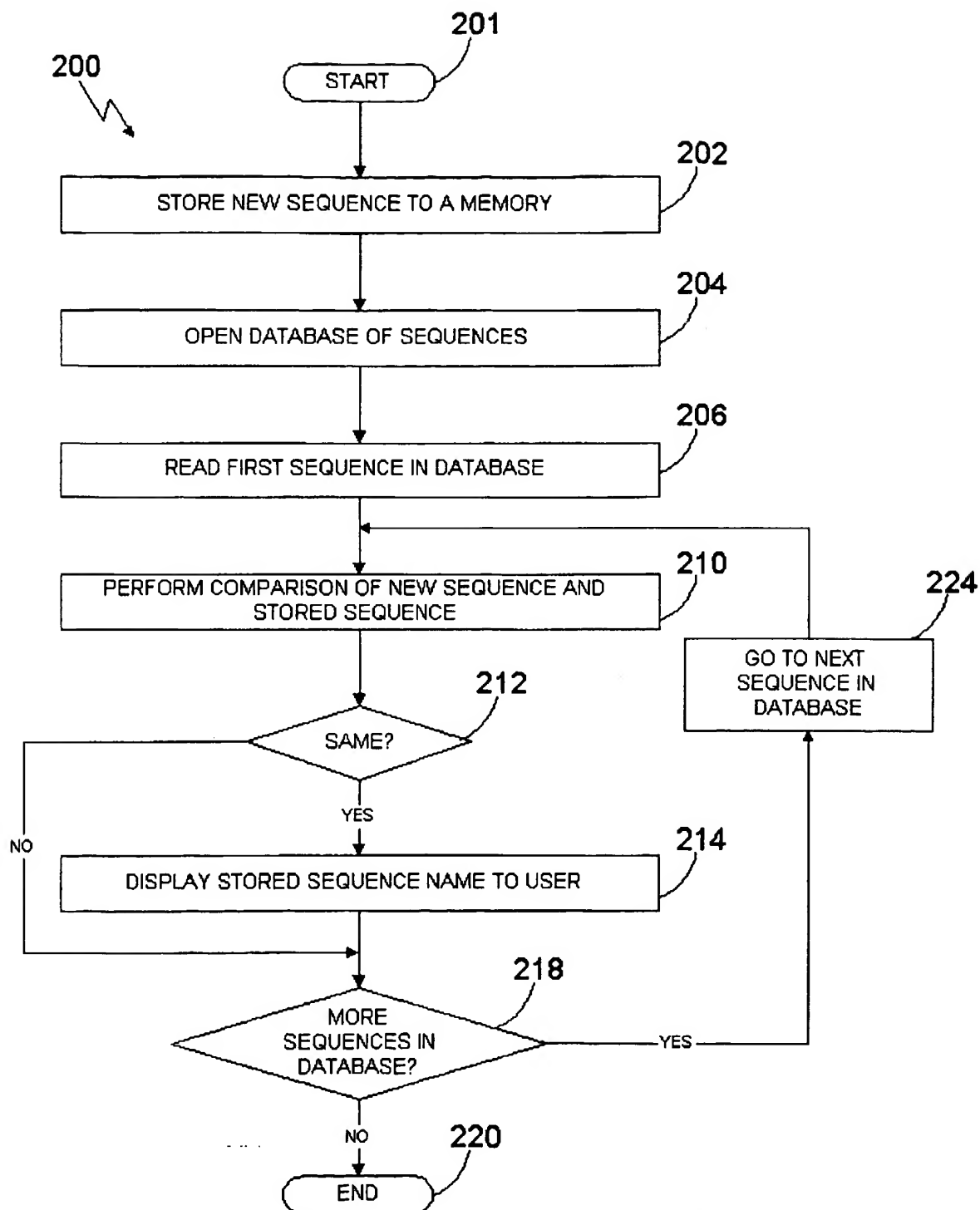


FIGURE 7

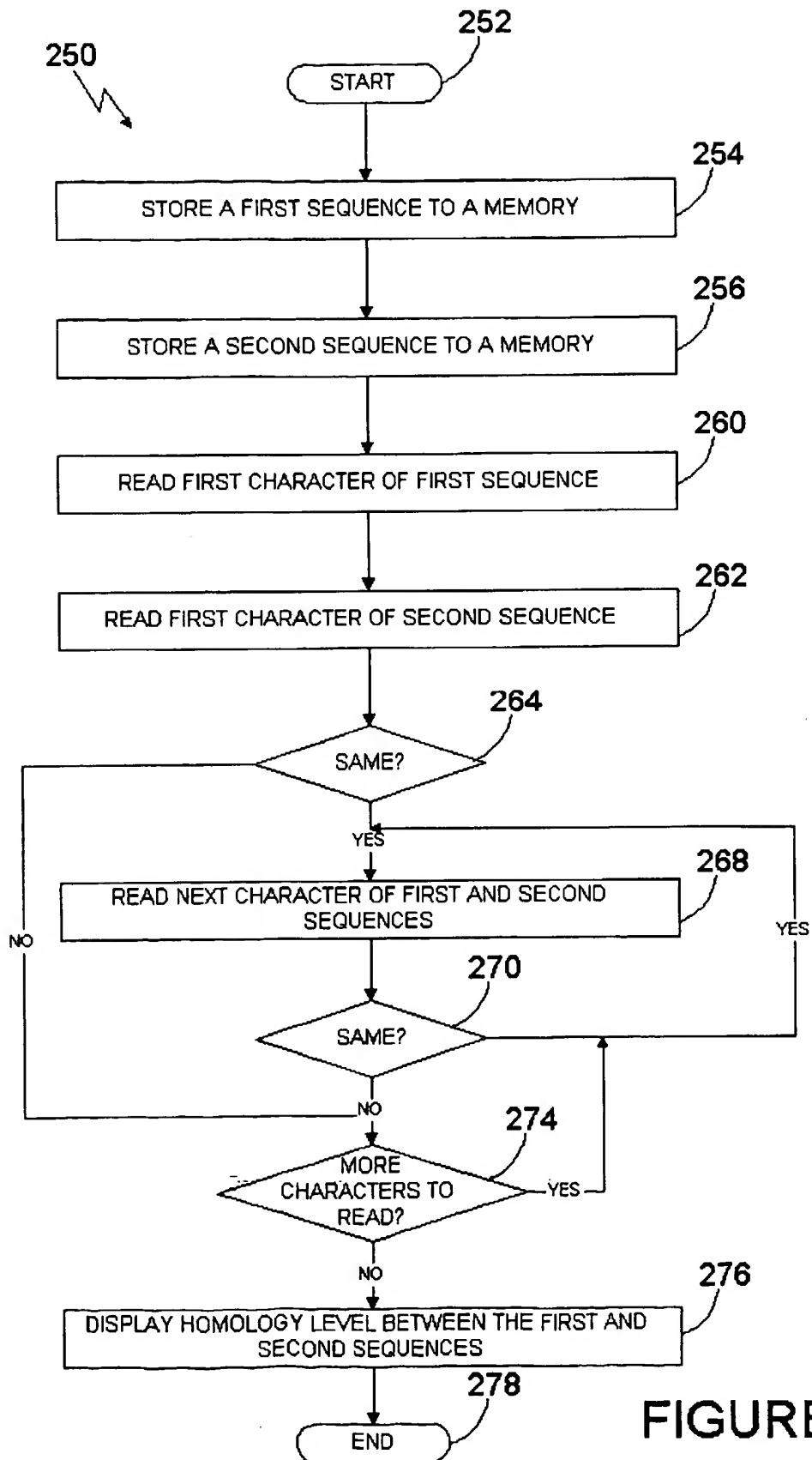


FIGURE 8

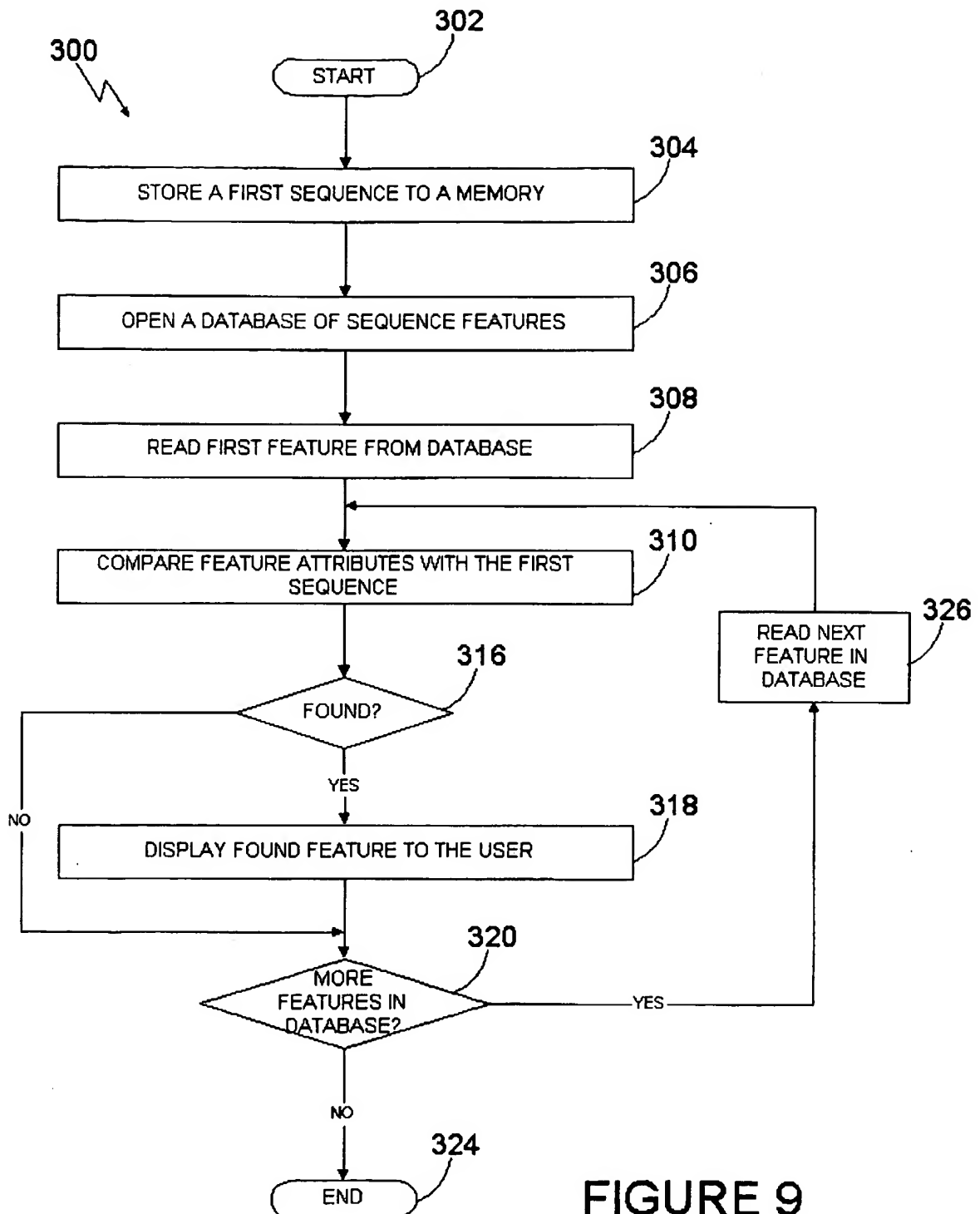


FIGURE 9

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<120> Complementary DNA's Encoding Proteins with Signal Peptides

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<150> 60/141,032

<151> 1999-06-25

<150> 09/469,099

<151> 1999-12-21

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<221> sig\_peptide

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<223> Von Heijne matrix  
score 4.1

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<222> 1392..1397

<220>

<221> polyA\_site

<222> 1432..1447

<220>

<221> misc\_feature

<222> 243,252,278,285,387,1429

<223> n=a, g, c or t

<400> 1

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agaaggggac	ccaaggggtc	ggccgcggcc	aaggccccta	ggaccgccgc	cccagctcac	120
gctgccgacg	gcagctatag	acattctgcg	tcagggtccg	gctcctggac	tttgcctttc	180
ccgagccctg	gaggtgggga	gaaaagggtc	accaattttt	aaaatccaaa	tatatctcat	240
ggntacagt	gnaagaactg	gccagagagt	ctggaagntt	tgggnttctg	gtcctggctg	300
tgccactgac	tcactgtgac	cttgggatct	tgtgctgtga	agacatttcc	caagtgcctc	360
atgttagcca	gcaaactcga	cccacanggc	ctggaaagag	gtgattgtta	ggttgcgcag	420
aggtgggtctt	atccagctca	gcttcccctg	ggaccaccgc	tgggacctga	ggcagaactg	480
gggtggactt	ggcctcctcc	atg gca cac	cgg ctg cag	ata cga ctg	ctg acg	533
		Met Ala His	Arg Leu Gln	Ile Arg Leu	Leu Thr	
		-240		-235		
tgg gat	gtg aag	gac acg	ctg ctc	agg ctc	cgc cac	ccc tta
Trp Asp	Val Lys	Asp Thr	Leu Leu	Arg Leu	Arg His	Pro Leu
	-230		-225		-220	
gcc tat	gcc acc	aag gcc	cgg gcc	cat ggg	ctg gag	gtg gag
Ala Tyr	Ala Thr	Lys Ala	Arg Ala	His Gly	Leu Glu	Val Glu
						Pro Ser
						629

2

```

-215          -210          -205
gcc ctg gaa caa ggc ttc agg cag gca tac agg gct cag agc cac agc      677
Ala Leu Glu Gln Gly Phe Arg Gln Ala Tyr Arg Ala Gln Ser His Ser
-200          -195          -190          -185
ttc ccc aac tac ggc ctg agc cac ggc cta acc tcc cgc cag tgg tgg      725
Phe Pro Asn Tyr Gly Leu Ser His Gly Leu Thr Ser Arg Gln Trp Trp
          -180          -175          -170
ctg gat gtg gtc ctg cag acc ttc cac ctg gcg ggt gtc cag gat gct      773
Leu Asp Val Val Leu Gln Thr Phe His Leu Ala Gly Val Gln Asp Ala
          -165          -160          -155
cag gct gta gcc ccc atc gct gaa cag ctt tat aaa gac ttc agc cac      821
Gln Ala Val Ala Pro Ile Ala Glu Gln Leu Tyr Lys Asp Phe Ser His
          -150          -145          -140
ccc tgc acc tgg cag gtg ttg gat ggg gct gag gac acc ctg agg gag      869
Pro Cys Thr Trp Gln Val Leu Asp Gly Ala Glu Asp Thr Leu Arg Glu
          -135          -130          -125
tgc cgc aca cgg ggt ctg aga ctg gca gtg atc tcc aac ttt gac cga      917
Cys Arg Thr Arg Gly Leu Arg Leu Ala Val Ile Ser Asn Phe Asp Arg
          -120          -115          -110          -105
cgg cta gag ggc atc ctg gag ggc ctt ggc ctg cgt gaa cac ttc gac      965
Arg Leu Glu Gly Ile Leu Glu Gly Leu Gly Leu Arg Glu His Phe Asp
          -100          -95          -90
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Phe Val Leu Thr Ser Glu Ala Ala Gly Trp Pro Lys Pro Asp Pro Arg
          -85          -80          -75
att ttc cag gag gcc ttg cgg ctt gct cat atg gaa cca gta gtg gca      1061
Ile Phe Gln Glu Ala Leu Arg Leu Ala His Met Glu Pro Val Val Ala
          -70          -65          -60
gcc cat gtt ggg gat aat tac ctc tgc gat tac cag ggg cct cgg gct      1109
Ala His Val Gly Asp Asn Tyr Leu Cys Asp Tyr Gln Gly Pro Arg Ala
          -55          -50          -45
gtg ggc atg cac agc ttc ctg gtg gtt ggc cca cag gca ctg gac ccc      1157
Val Gly Met His Ser Phe Leu Val Val Gly Pro Gln Ala Leu Asp Pro
          -40          -35          -30          -25
gtg gtc agg gat tct gta cct aaa gaa cac atc ctc ccc tct ctg gcc      1205
Val Val Arg Asp Ser Val Pro Lys Glu His Ile Leu Pro Ser Leu Ala
          -20          -15          -10
cat ctc ctg cct gcc ctt gac tgc cta gag ggc tca act cca ggg ctt      1253
His Leu Leu Pro Ala Leu Asp Cys Leu Glu Gly Ser Thr Pro Gly Leu
          -5          1          5
tgaggccagt gagggaaagtg gctgggccct aggccatgga gaaaacctta aacaaacctt      1313
ggagacaggg agcccccttct ttctccacag ctctggacct tccccctctt ccctgcgggc      1373
tttgtcacct actgtgataa taaagcagtg agtgetgagc tctcaccctt ccccnccaa      1433
aaaaaaaaaa aaaa      1447

```

&lt;210&gt; 2

&lt;211&gt; 251

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -243..-1

&lt;400&gt; 2

```

Met Ala His Arg Leu Gln Ile Arg Leu Leu Thr Trp Asp Val Lys Asp
          -240          -235          -230
Thr Leu Leu Arg Leu Arg His Pro Leu Gly Glu Ala Tyr Ala Thr Lys
          -225          -220          -215
Ala Arg Ala His Gly Leu Glu Val Glu Pro Ser Ala Leu Glu Gln Gly
          -210          -205          -200
Phe Arg Gln Ala Tyr Arg Ala Gln Ser His Ser Phe Pro Asn Tyr Gly

```



3

```

-195          -190          -185          -180
Leu Ser His Gly Leu Thr Ser Arg Gln Trp Trp Leu Asp Val Val Leu
          -175          -170          -165
Gln Thr Phe His Leu Ala Gly Val Gln Asp Ala Gln Ala Val Ala Pro
          -160          -155          -150
Ile Ala Glu Gln Leu Tyr Lys Asp Phe Ser His Pro Cys Thr Trp Gln
          -145          -140          -135
Val Leu Asp Gly Ala Glu Asp Thr Leu Arg Glu Cys Arg Thr Arg Gly
          -130          -125          -120
Leu Arg Leu Ala Val Ile Ser Asn Phe Asp Arg Arg Leu Glu Gly Ile
          -115          -110          -105          -100
Leu Glu Gly Leu Gly Leu Arg Glu His Phe Asp Phe Val Leu Thr Ser
          -95          -90          -85
Glu Ala Ala Gly Trp Pro Lys Pro Asp Pro Arg Ile Phe Gln Glu Ala
          -80          -75          -70
Leu Arg Leu Ala His Met Glu Pro Val Val Ala Ala His Val Gly Asp
          -65          -60          -55
Asn Tyr Leu Cys Asp Tyr Gln Gly Pro Arg Ala Val Gly Met His Ser
          -50          -45          -40
Phe Leu Val Val Gly Pro Gln Ala Leu Asp Pro Val Val Arg Asp Ser
          -35          -30          -25          -20
Val Pro Lys Glu His Ile Leu Pro Ser Leu Ala His Leu Leu Pro Ala
          -15          -10          -5
Leu Asp Cys Leu Glu Gly Ser Thr Pro Gly Leu
      1              5

```

```

<210> 3
<211> 1448
<212> DNA
<213> Homo sapiens

```

```

<220>
<221> CDS
<222> 131..490

<220>
<221> sig_peptide
<222> 131..301
<223> Von Heijne matrix
      score 5.31
      seq AIALATVLFLLIGA/FL

```

```

<220>
<221> polyA_signal
<222> 1411..1416

```

```

<220>
<221> polyA_site
<222> 1434..1448

```

```

<400> 3
ctgatcccg c tggggccgg ctgagtggca cttaagcggg ccatgccatg caaccttggg      60
cgctgccaac cgtgggagag ctctgggtgt gcgggcggcc tcgcgcggcg ctccgctgtg      120
tcagcgtgtt atg atg ccg tcc cgt acc aac ctg gct act gga atc ccc      169
      Met Met Pro Ser Arg Thr Asn Leu Ala Thr Gly Ile Pro
          -55          -50          -45
agt agt aaa gtg aaa tat tca agg ctc tcc agc aca gac gat ggc tac      217
Ser Ser Lys Val Lys Tyr Ser Arg Leu Ser Ser Thr Asp Asp Gly Tyr
          -40          -35          -30
att gac ctt cag ttt aag aaa acc cct cct aag atc cct tat aag gcc      265
Ile Asp Leu Gln Phe Lys Lys Thr Pro Pro Lys Ile Pro Tyr Lys Ala
          -25          -20          -15

```

4

```

atc gca ctt gcc act gtg ctg ttt ttg att ggc gcc ttt ctc att att      313
Ile Ala Leu Ala Thr Val Leu Phe Leu Ile Gly Ala Phe Leu Ile Ile
      -10                      -5                      1
ata ggc tcc ctc ctg ctg tca ggc tac atc agc aaa ggg ggg gca gac      361
Ile Gly Ser Leu Leu Leu Ser Gly Tyr Ile Ser Lys Gly Gly Ala Asp
5                      10                      15                      20
cgg gcc gtt cca gtg ctg atc att ggc att ctg gtg ttc cta ccc gga      409
Arg Ala Val Pro Val Leu Ile Ile Gly Ile Leu Val Phe Leu Pro Gly
      25                      30                      35
ttt tac cac ctg cgc atc gct tac tat gca tcc aaa ggc tac cgt ggt      457
Phe Tyr His Leu Arg Ile Ala Tyr Tyr Ala Ser Lys Gly Tyr Arg Gly
      40                      45                      50
tac tcc tat gat gac att cca gac ttt gat gac tagcaccac cccatagctg      510
Tyr Ser Tyr Asp Asp Ile Pro Asp Phe Asp Asp
      55                      60
aggaggagtc acagtggaac tgtcccagct ttaagatatc tagcagaaac tatagctgag      570
gactaaggaa ttctgcagct tgcagatggt taagaaaata atggccagat tttttgggtc      630
cttcccaaag atgttaagtg aacctacagt tagctaatta ggacaagctc tatttttcat      690
ccctgggccc tgacaagtgt ttccacagga atatgtatca tggagaata gaggttattc      750
tgtaatggaa aagtgttgcc tgccaccacc ctctgtagag ctgagcattt cttttaaata      810
gtcttcattg ccaatttggt cttgtagcaa atggaacaat gtggtatggc taatttctta      870
ttattaagta atttatttta aaaatatctg agtatattat cctgtacact tatccctacc      930
ttcatgttcc agtggaagac cttagtataa tcaaagatca gtgagttcat ctgtaattatt      990
ttttttactt gctttcttac tgacagcaac caggaatttt tttatcctgc agagcaagtt      1050
ttcaaaatgt aaatacttcc tctgtttaac agtccttgga ccattctgat ccagttcacc      1110
agtaggttgg acagcatata atttgcata ttttgtccct tgtaaatcaa gatgttctgc      1170
agattattcc tttaacggcc ggacttttgg ctgtttccta atgaaacatg tagtggttat      1230
tatttagagt ttatagcogt attgctagca ccttgtagta tgtcatcatt ctgctcatga      1290
ttccaaggat cagcctggat gcctagagga ctagatcacc ttagtttgat tctatttttt      1350
agcttgcaaa aagtgactta tattccaaaag aaattaaaat gttgaaatcc aaatcctaga      1410
aataaaatga gttaacttca aacaaaaaaa aaaaaaaa      1448

```

<210> 4  
 <211> 120  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SIGNAL  
 <222> -57...-1

```

<400> 4
Met Met Pro Ser Arg Thr Asn Leu Ala Thr Gly Ile Pro Ser Ser Lys
      -55                      -50                      -45
Val Lys Tyr Ser Arg Leu Ser Ser Thr Asp Asp Gly Tyr Ile Asp Leu
      -40                      -35                      -30
Gln Phe Lys Lys Thr Pro Pro Lys Ile Pro Tyr Lys Ala Ile Ala Leu
      -25                      -20                      -15                      -10
Ala Thr Val Leu Phe Leu Ile Gly Ala Phe Leu Ile Ile Ile Gly Ser
      -5                      1                      5
Leu Leu Leu Ser Gly Tyr Ile Ser Lys Gly Gly Ala Asp Arg Ala Val
      10                      15                      20
Pro Val Leu Ile Ile Gly Ile Leu Val Phe Leu Pro Gly Phe Tyr His
      25                      30                      35
Leu Arg Ile Ala Tyr Tyr Ala Ser Lys Gly Tyr Arg Gly Tyr Ser Tyr
      40                      45                      50                      55
Asp Asp Ile Pro Asp Phe Asp Asp
      60

```

<210> 5  
 <211> 1515  
 <212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> 165..842

<220>

<221> sig\_peptide

<222> 165..251

<223> Von Heijne matrix

score 7.01

seq LASFAALVLVCRO/R

<220>

<221> polyA\_signal

<222> 1474..1479

<220>

<221> polyA\_site

<222> 1500..1515

<400> 5

```

agtcgcggga tgcgcccggg agccacagcc tgaggccctc aggtctctgc aggtgtcgtg      60
gaggaacctc gcacctgcca tcctcttccc caatttgcca cttccagcag ctttagccca      120
tgaggaggat gtgaccggga ctgagtcagg agccctctgg aagc atg gag act gtg      176
                               Met Glu Thr Val
gtg att gtt gcc ata ggt gtg ctg gcc acc atc ttt ctg gct tcg ttt      224
Val Ile Val Ala Ile Gly Val Leu Ala Thr Ile Phe Leu Ala Ser Phe
-25                               -20                               -15                               -10
gca gcc ttg gtg ctg gtt tgc agg cag cgc tac tgc cgg ccg cga gac      272
Ala Ala Leu Val Leu Val Cys Arg Gln Arg Tyr Cys Arg Pro Arg Asp
                               -5                               1                               5
ctg ctg cag cgc tat gat tct aag ccc att gtg gac ctc att ggt gcc      320
Leu Leu Gln Arg Tyr Asp Ser Lys Pro Ile Val Asp Leu Ile Gly Ala
                               10                               15                               20
atg gag acc cag tct gag ccc tct gag tta gaa ctg gac gat gtc gtt      368
Met Glu Thr Gln Ser Glu Pro Ser Glu Leu Glu Leu Asp Asp Val Val
                               25                               30                               35
atc acc aac ccc cac att gag gcc att ctg gag aat gaa gac tgg atc      416
Ile Thr Asn Pro His Ile Glu Ala Ile Leu Glu Asn Glu Asp Trp Ile
40                               45                               50                               55
gaa gat gcc tcg ggt ctc atg tcc cac tgc att gcc atc ttg aag att      464
Glu Asp Ala Ser Gly Leu Met Ser His Cys Ile Ala Ile Leu Lys Ile
                               60                               65                               70
tgt cac act ctg aca gag aag ctt gtt gcc atg aca atg ggc tct ggg      512
Cys His Thr Leu Thr Glu Lys Leu Val Ala Met Thr Met Gly Ser Gly
                               75                               80                               85
gcc aag atg aag act tca gcc agt gtc agc gac atc att gtg gtg gcc      560
Ala Lys Met Lys Thr Ser Ala Ser Val Ser Asp Ile Ile Val Val Ala
                               90                               95                               100
aag cgg atc agc ccc agg gtg gat gat gtt gtg aag tcg atg tac cct      608
Lys Arg Ile Ser Pro Arg Val Asp Asp Val Val Lys Ser Met Tyr Pro
                               105                               110                               115
ccg ttg gac ccc aaa ctc ctg gac gca cgg acg act gcc ctg ctc ctg      656
Pro Leu Asp Pro Lys Leu Leu Asp Ala Arg Thr Thr Ala Leu Leu Leu
120                               125                               130                               135
tct gtc agt cac ctg gtg ctg gtg aca agg aat gcc tgc cat ctg acg      704
Ser Val Ser His Leu Val Leu Val Thr Arg Asn Ala Cys His Leu Thr
                               140                               145                               150
gga ggc ctg gac tgg att gac cag tct ctg tcg gct gct gag gag cat      752
Gly Gly Leu Asp Trp Ile Asp Gln Ser Leu Ser Ala Ala Glu Glu His
                               155                               160                               165

```

6

```

ttg gaa gtc ctt cga gaa gca gcc cta gct tct gag cca gat aaa ggc      800
Leu Glu Val Leu Arg Glu Ala Ala Leu Ala Ser Glu Pro Asp Lys Gly
      170                      175                      180
ctc cca ggc cct gaa ggc ttc ctg cag gag cag tct gca att      842
Leu Pro Gly Pro Glu Gly Phe Leu Gln Glu Gln Ser Ala Ile
      185                      190                      195
tagtgccctac aggccagcag ctagccatga agggccctgc cgccatccct ggatggctca      902
gcttagcctt ctactttttc ctatagagtt agttgtttctc cacggctgga gagttcagct      962
gtgtgtgcat agtaaagcag gagatccccg tcagtttatg cctcttttgc agttgcaaac     1022
tgtggctggt gagtggcagt ctaatactac agttagggga gatgccattc actctctgca     1082
agaggagtat tgaaaactgg tggactgtca gctttattta gctcacctag tgttttcaag     1142
aaaattgagc caccgtctaa gaaatcaaga ggtttcacat taaaattaga atttctggcc     1202
tctctcgatc ggtcagaatg tgtggcaatt ctgatctgca ttttcagaag aggacaatca     1262
attgaaacta agtaggggtt tcttcttttg gcaagacttg tactctctca cctggcctgt     1322
ttcattttatt tgtattatct gcctgggtccc tgaggcgtct gggctctctc tctcccttgc     1382
aggtttgggt ttgaagctga ggaactacaa agttgatgat ttctttttta tctttatgcc     1442
tgcaatttta cctagctacc actaggtgga tagtaaattt atacttatgt ttcccccaaa     1502
aaaaaaaaaaa aaa                                                    1515

```

<210> 6  
 <211> 226  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SIGNAL  
 <222> -29...-1

```

<400> 6
Met Glu Thr Val Val Ile Val Ala Ile Gly Val Leu Ala Thr Ile Phe
      -25                      -20                      -15
Leu Ala Ser Phe Ala Ala Leu Val Leu Val Cys Arg Gln Arg Tyr Cys
      -10                      -5                      1
Arg Pro Arg Asp Leu Leu Gln Arg Tyr Asp Ser Lys Pro Ile Val Asp
      5                      10                      15
Leu Ile Gly Ala Met Glu Thr Gln Ser Glu Pro Ser Glu Leu Glu Leu
      20                      25                      30                      35
Asp Asp Val Val Ile Thr Asn Pro His Ile Glu Ala Ile Leu Glu Asn
      40                      45                      50
Glu Asp Trp Ile Glu Asp Ala Ser Gly Leu Met Ser His Cys Ile Ala
      55                      60                      65
Ile Leu Lys Ile Cys His Thr Leu Thr Glu Lys Leu Val Ala Met Thr
      70                      75                      80
Met Gly Ser Gly Ala Lys Met Lys Thr Ser Ala Ser Val Ser Asp Ile
      85                      90                      95
Ile Val Val Ala Lys Arg Ile Ser Pro Arg Val Asp Asp Val Val Lys
      100                      105                      110                      115
Ser Met Tyr Pro Pro Leu Asp Pro Lys Leu Leu Asp Ala Arg Thr Thr
      120                      125                      130
Ala Leu Leu Leu Ser Val Ser His Leu Val Leu Val Thr Arg Asn Ala
      135                      140                      145
Cys His Leu Thr Gly Gly Leu Asp Trp Ile Asp Gln Ser Leu Ser Ala
      150                      155                      160
Ala Glu Glu His Leu Glu Val Leu Arg Glu Ala Ala Leu Ala Ser Glu
      165                      170                      175
Pro Asp Lys Gly Leu Pro Gly Pro Glu Gly Phe Leu Gln Glu Gln Ser
      180                      185                      190                      195
Ala Ile

```

<210> 7  
 <211> 1918  
 <212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> 238..612

<220>

<221> sig\_peptide

<222> 238..348

<223> Von Heijne matrix

score 9.4

seq LLCCVLSASQLSS/QD

<220>

<221> polyA\_signal

<222> 1885..1890

<220>

<221> polyA\_site

<222> 1905..1918

<220>

<221> misc\_feature

<222> 945,1624

<223> n=a, g, c or t

<400> 7

aaaaatctaa gcgacttcga tgccaaggaa gttgtgtaaa tgtgcacgcg ctacaccaca	60
cccagggtgg aaaccacagt tgcagagtca ttaaacaatc aattgtttgt ttaacatctg	120
tgataggcag ctttccttct tttcaacagt gatacctacg aaaatcaaaa taaatgcaag	180
ctgagggttt gtgctcactg aaagggtgt caaccccaga aggccgacac aaaaaaa	237
atg gta tgt gaa gat gca ccg tct ttt caa atg gcc tgg gag agt caa	285
Met Val Cys Glu Asp Ala Pro Ser Phe Gln Met Ala Trp Glu Ser Gln	
-35 -30 -25	
atg gcc tgg gag agg ggg cct gcc ctt ctc tgc tgt gtc ctt tcg gct	333
Met Ala Trp Glu Arg Gly Pro Ala Leu Leu Cys Cys Val Leu Ser Ala	
-20 -15 -10	
tcc cag ttg agc tcc caa gac cag gac cca ctg ggg cat ata aaa tct	381
Ser Gln Leu Ser Ser Gln Asp Gln Asp Pro Leu Gly His Ile Lys Ser	
-5 1 5 10	
ctg ctg tat cct ttc ggc ttc cca gtt gag ctc cca aga cca gga ccc	429
Leu Leu Tyr Pro Phe Gly Phe Pro Val Glu Leu Pro Arg Pro Gly Pro	
15 20 25	
act ggg gca tat aaa aaa gtc aaa aat caa aat caa aca aca agt tct	477
Thr Gly Ala Tyr Lys Lys Val Lys Asn Gln Asn Gln Thr Thr Ser Ser	
30 35 40	
gag tta ctt agg aaa cag act tcg cat ttc aat cag aga ggc cac aga	525
Glu Leu Leu Arg Lys Gln Thr Ser His Phe Asn Gln Arg Gly His Arg	
45 50 55	
gca agg tct aaa ctt ctg gct tct aga caa att cct gat aga aca ttt	573
Ala Arg Ser Lys Leu Leu Ala Ser Arg Gln Ile Pro Asp Arg Thr Phe	
60 65 70 75	
aaa tgt ggg aag tgg ctt ccc cag gtc cca tcc cct gtt tagggataga	622
Lys Cys Gly Lys Trp Leu Pro Gln Val Pro Ser Pro Val	
80 85	
gttgatatca tttttatagt tgccatgtat gcctctgcct gaattttttt aattgacttt	682
tgagcttttg agattgcacg agggagaaca aggcctttgc tgttgtggat aggaaagact	742
taacctaaaa ttaaaccagc aagaaagcat tagtaaaaaat ctaacaatat gaagggctct	802
tatgagtcac ttttttcaaa agatgaaaac tccagaaacg cacaggaacg aaatacctcc	862
cagaacatg aagcaatcat cgaagactca ctggtaatat ttttaaaaag tatacagatc	922
aaagcaaaaa gaagccatgt gtnaacaaaag agaaatgtgc aaatatTTTT taaggcagta	982
ttaagtgcaa gaggagtaac atgaaataaa cattcttttca catggctact gggaatataa	1042

```

atttcgctcc agaaaggccg tagcagtttg acgataggtg gcaaaacctt aagatttgtgt 1102
actggggccc agaattttta tttctaggaa tgtatcctga ggaaattatc cgagatcccc 1162
acaaactgca atgttttagga attgtcctta tagcattgca tacacaagaa aaacagagaa 1222
aagcctgata cctgtcagtg gaaaaggggt tcaatgaatt acggtgtgtc tgcagagggc 1282
ttttatgaca ttaaaaattg ttgaacaacg gccaggcaca gtggctcatg cctgtaatcc 1342
taacactttg ggaggccaag gtgggaagat tgcctgagct caggagtttg agaccagcct 1402
gggcaacacg gtgaaacccc gtctctacta aaatacaaaa aattagccgg gcgtcgagc 1462
atgcgcctgt agtcccagct gctcaggagg ctgaggcagg agaattgatt gaaccgggga 1522
ggcagagggtt gactgagct gagattaagc caccgcactc cagcctgggc gacagagcaa 1582
gattccgttc ccaagaaaaa aaaattgttc aacaataagg gncaaaggga gagaatcata 1642
acatctgatt aaacagaaaa agcaagattt ttaaaactaa ctatataagg atggtcccag 1702
ctgtgtcaaa aggaagcttg tttgtaatac gtgtgcataa aaattaaata gaggtgaaca 1762
caattatttt aaggcagtta aattatctct gtattgtgaa ctaagacttt ctagaatttt 1822
acttattcat tctgtactta aattttttct aatgaacaca tatacttttg taatcagaaa 1882
atattaaatg catgtatttt tcaaaaaaaaa aaaaaa 1918

```

```

<210> 8
<211> 125
<212> PRT
<213> Homo sapiens

```

```

<220>
<221> SIGNAL
<222> -37..-1

```

```

<400> 8
Met Val Cys Glu Asp Ala Pro Ser Phe Gln Met Ala Trp Glu Ser Gln
      -35                      -30                      -25
Met Ala Trp Glu Arg Gly Pro Ala Leu Leu Cys Cys Val Leu Ser Ala
      -20                      -15                      -10
Ser Gln Leu Ser Ser Gln Asp Gln Asp Pro Leu Gly His Ile Lys Ser
      -5                      1                      5                      10
Leu Leu Tyr Pro Phe Gly Phe Pro Val Glu Leu Pro Arg Pro Gly Pro
      15                      20                      25
Thr Gly Ala Tyr Lys Lys Val Lys Asn Gln Asn Gln Thr Thr Ser Ser
      30                      35                      40
Glu Leu Leu Arg Lys Gln Thr Ser His Phe Asn Gln Arg Gly His Arg
      45                      50                      55
Ala Arg Ser Lys Leu Leu Ala Ser Arg Gln Ile Pro Asp Arg Thr Phe
      60                      65                      70                      75
Lys Cys Gly Lys Trp Leu Pro Gln Val Pro Ser Pro Val
      80                      85

```

```

<210> 9
<211> 852
<212> DNA
<213> Homo sapiens

```

```

<220>
<221> CDS
<222> 229..735

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```

<220>
<221> sig_peptide
<222> 229..492
<223> Von Heijne matrix
      score 6.7
      seq VFALSSFLNKASA/VY

```

```

<220>
<221> polyA_signal
<222> 816..821

```

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 841..852

&lt;400&gt; 9

```

aatgactggc agtggcatca gcgatggcgg ctgcgtcggg gtcggttctg cagcgtgta      60
tcgtgtcgcc ggcagggagg catagcgcoct ctctgatctt cctgcatggc tcaggtgatt    120
ctggacaagg attaagaatg tggatcaagc aggttttttaa atcaagattt aacattccaa    180
cacataaaaa ttatttatcc aacagctcct cccagatcat atactcct atg aaa gga    237
                                     Met Lys Gly
gga atc tcc aat gta tgg ttt gac aga ttt aaa ata acc aat gac tgc      285
Gly Ile Ser Asn Val Trp Phe Asp Arg Phe Lys Ile Thr Asn Asp Cys
-85          -80          -75          -70
cca gaa cac ctt gaa tca att gat gtc atg tgt caa gtg ctt act gat      333
Pro Glu His Leu Glu Ser Ile Asp Val Met Cys Gln Val Leu Thr Asp
-65          -60          -55
ttg att gat gaa gaa gta aaa agt ggc atc aag aag aac agg ata tta      381
Leu Ile Asp Glu Glu Val Lys Ser Gly Ile Lys Lys Asn Arg Ile Leu
-50          -45          -40
ata gga gga ttc tct atg gga gga tgc atg gca atg cat tta gca tat      429
Ile Gly Gly Phe Ser Met Gly Gly Cys Met Ala Met His Leu Ala Tyr
-35          -30          -25
aga aat cat caa gat gtg gca gga gta ttt gct ctt tct agt ttt ctg      477
Arg Asn His Gln Asp Val Ala Gly Val Phe Ala Leu Ser Ser Phe Leu
-20          -15          -10
aat aaa gca tct gct gtt tac cag gct ctt cag aag agt aat ggt gta      525
Asn Lys Ala Ser Ala Val Tyr Gln Ala Leu Gln Lys Ser Asn Gly Val
-5          1          5          10
ctt cct gaa tta ttt cag tgt cat ggt act gca gat gag tta gtt ctt      573
Leu Pro Glu Leu Phe Gln Cys His Gly Thr Ala Asp Glu Leu Val Leu
15          20          25
cat tct tgg gca gaa gag aca aac tca atg tta aaa tct cta gga gtg      621
His Ser Trp Ala Glu Glu Thr Asn Ser Met Leu Lys Ser Leu Gly Val
30          35          40
acc acg aag ttt cat agt ttt cca aat gtt tac cat gag cta agc aaa      669
Thr Thr Lys Phe His Ser Phe Pro Asn Val Tyr His Glu Leu Ser Lys
45          50          55
act gag tta gac ata ttg aag tta tgg att ctt aca aag ctg cca gga      717
Thr Glu Leu Asp Ile Leu Lys Leu Trp Ile Leu Thr Lys Leu Pro Gly
60          65          70          75
gaa atg gaa aaa caa aaa tgaatgaatc aagagtgatt tgttaatgta      765
Glu Met Glu Lys Gln Lys
80
agtgtaatgt ctttgtgaaa agtgattttt actgccaaat tataatgata attaaaaatat    825
taagaaatag caaaaaaaaaa aaaaaaa      852

```

&lt;210&gt; 10

&lt;211&gt; 169

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -88..-1

&lt;400&gt; 10

```

Met Lys Gly Gly Ile Ser Asn Val Trp Phe Asp Arg Phe Lys Ile Thr
-85          -80          -75
Asn Asp Cys Pro Glu His Leu Glu Ser Ile Asp Val Met Cys Gln Val
-70          -65          -60
Leu Thr Asp Leu Ile Asp Glu Glu Val Lys Ser Gly Ile Lys Lys Asn

```

10

```

      -55              -50              -45
Arg Ile Leu Ile Gly Gly Phe Ser Met Gly Gly Cys Met Ala Met His
-40              -35              -30              -25
Leu Ala Tyr Arg Asn His Gln Asp Val Ala Gly Val Phe Ala Leu Ser
      -20              -15              -10
Ser Phe Leu Asn Lys Ala Ser Ala Val Tyr Gln Ala Leu Gln Lys Ser
      -5              1              5
Asn Gly Val Leu Pro Glu Leu Phe Gln Cys His Gly Thr Ala Asp Glu
10              15              20
Leu Val Leu His Ser Trp Ala Glu Glu Thr Asn Ser Met Leu Lys Ser
25              30              35              40
Leu Gly Val Thr Thr Lys Phe His Ser Phe Pro Asn Val Tyr His Glu
      45              50              55
Leu Ser Lys Thr Glu Leu Asp Ile Leu Lys Leu Trp Ile Leu Thr Lys
      60              65              70
Leu Pro Gly Glu Met Glu Lys Gln Lys
      75              80

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<210> 11  
 <211> 1602  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> 24..1004

<220>  
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 <222> 24..170  
 <223> Von Heijne matrix  
 score 5.6  
 seq ACLSLGFFSLLWL/QL

<220>  
 <221> polyA\_site  
 <222> 1586..1602

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<400> 11
atgcgccgcc gcctctccgc acg atg ttc ccc tcg cgg agg aaa gcg gcg cag      53
                                Met Phe Pro Ser Arg Arg Lys Ala Ala Gln
                                -45              -40

ctg ccc tgg gag gac ggc agg tcc ggg ttg ctc tcc ggc ggc ctc cct      101
Leu Pro Trp Glu Asp Gly Arg Ser Gly Leu Leu Ser Gly Gly Leu Pro
      -35              -30              -25
cgg aag tgt tcc gtc ttc cac ctg ttc gtg gcc tgc ctc tcg ctg ggc      149
Arg Lys Cys Ser Val Phe His Leu Phe Val Ala Cys Leu Ser Leu Gly
      -20              -15              -10
ttc ttc tcc cta ctc tgg ctg cag ctc agc tgc tct ggg gac gtg gcc      197
Phe Phe Ser Leu Leu Trp Leu Gln Leu Ser Cys Ser Gly Asp Val Ala
      -5              1              5
cgg gca gtc agg gga caa ggg cag gag acc tcg ggc cct ccc cgt gcc      245
Arg Ala Val Arg Gly Gln Gly Gln Glu Thr Ser Gly Pro Pro Arg Ala
10              15              20              25
tgc ccc cca gag ccg ccc cct gag cac tgg gaa gaa gac gca tcc tgg      293
Cys Pro Pro Glu Pro Pro Glu His Trp Glu Glu Asp Ala Ser Trp
      30              35              40
ggc ccc cac cgc ctg gca gtg ctg gtg ccc ttc cgc gaa cgc ttc gag      341
Gly Pro His Arg Leu Ala Val Leu Val Pro Phe Arg Glu Arg Phe Glu
      45              50              55
gag ctc ctg gtc ttc gtg ccc cac atg cgc cgc ttc ctg agc agg aag      389
Glu Leu Leu Val Phe Val Pro His Met Arg Arg Phe Leu Ser Arg Lys

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11

60	65	70	
aag atc cgg cac cac atc tac gtg ctc aac cag gtg gac cac ttc agg			437
Lys Ile Arg His His Ile Tyr Val Leu Asn Gln Val Asp His Phe Arg			
75	80	85	
ttc aac cgg gca gcg ctc atc aac gtg ggc ttc ctg gag agc agc aac			485
Phe Asn Arg Ala Ala Leu Ile Asn Val Gly Phe Leu Glu Ser Ser Asn			
90	95	100	105
agc acg gac tac att gcc atg cac gac gtt gac ctg ctc cct ctc aac			533
Ser Thr Asp Tyr Ile Ala Met His Asp Val Asp Leu Leu Pro Leu Asn			
110	115	120	
gag gag ctg gac tat ggc ttt cct gag gct ggg ccc ttc cac gtg gcc			581
Glu Glu Leu Asp Tyr Gly Phe Pro Glu Ala Gly Pro Phe His Val Ala			
125	130	135	
tcc ccg gag ctc cac cct ctc tac cac tac aag acc tat gtc ggc ggc			629
Ser Pro Glu Leu His Pro Leu Tyr His Tyr Lys Thr Tyr Val Gly Gly			
140	145	150	
atc ctg ctg ctc tcc aag cag cac tac cgg ctg tgc aat ggg atg tcc			677
Ile Leu Leu Leu Ser Lys Gln His Tyr Arg Leu Cys Asn Gly Met Ser			
155	160	165	
aac cgc ttc tgg ggc tgg ggc cgc gag gac gac gag ttc tac cgg cgc			725
Asn Arg Phe Trp Gly Trp Gly Arg Glu Asp Asp Glu Phe Tyr Arg Arg			
170	175	180	185
att aag gga gct ggg ctc cag ctt ttc cgc ccc tcg gga atc aca act			773
Ile Lys Gly Ala Gly Leu Gln Leu Phe Arg Pro Ser Gly Ile Thr Thr			
190	195	200	
ggg tac aag aca ttt cgc cac ctg cat gac cca gcc tgg cgg aag agg			821
Gly Tyr Lys Thr Phe Arg His Leu His Asp Pro Ala Trp Arg Lys Arg			
205	210	215	
gac cag aag cgc atc gca gct caa aaa cag gag cag ttc aag gtg gac			869
Asp Gln Lys Arg Ile Ala Ala Gln Lys Gln Glu Gln Phe Lys Val Asp			
220	225	230	
agg gag gga ggc ctg aac act gtg aag tac cat gtg gct tcc cgc act			917
Arg Glu Gly Gly Leu Asn Thr Val Lys Tyr His Val Ala Ser Arg Thr			
235	240	245	
gcc ctg tct gtg ggc ggg gcc ccc tgc act gtc ctc aac atc atg ttg			965
Ala Leu Ser Val Gly Gly Ala Pro Cys Thr Val Leu Asn Ile Met Leu			
250	255	260	265
gac tgt gac aag acc gcc aca ccc tgg tgc aca ttc agc tgagctggat			1014
Asp Cys Asp Lys Thr Ala Thr Pro Trp Cys Thr Phe Ser			
270	275		
ggacagtgag gaagcctgta cctacaggcc atattgctca ggctcaggac aaggcctcag			1074
gtcgtggggc cagctctgac aggatgtgga gtggccagga ccaagacagc aagctacgca			1134
attgcagcca cccggccgcc aaggcaggct tgggctgggc caggacacgt ggggtgcctg			1194
ggacgctgct tgccatgcac agtgatcaga gagaggctgg ggtgtgtcct gtccgggacc			1254
ccccctgcct tcctgctcac cctaactctga cctccttcac gtgcccaggc ctgtgggtag			1314
tggggagggc tgaacaggac aacctctcat ccccccaact tttgttcctt cctgctgggc			1374
tgccctcgtgc agagacacag tgtaggggcc atgcagctgg ctaggtggc agttggcct			1434
ggtgaggggtt aggacttcag aaaccagagc acaagcccca cagaggggga acagccagca			1494
ccgctctagc tgggtgtgac catgccgaa tgtgggccta gtgttgccag atcttctgat			1554
ttttcgaaag aaactagaat gctggattct caaaaaaaaa aaaaaaaaa			1602

&lt;210&gt; 12

&lt;211&gt; 327

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -49..-1

&lt;400&gt; 12

Met Phe Pro Ser Arg Arg Lys Ala Ala Gln Leu Pro Trp Glu Asp Gly

12

```

          -45          -40          -35
Arg Ser Gly Leu Leu Ser Gly Gly Leu Pro Arg Lys Cys Ser Val Phe
          -30          -25          -20
His Leu Phe Val Ala Cys Leu Ser Leu Gly Phe Phe Ser Leu Leu Trp
          -15          -10          -5
Leu Gln Leu Ser Cys Ser Gly Asp Val Ala Arg Ala Val Arg Gly Gln
   1           5           10           15
Gly Gln Glu Thr Ser Gly Pro Pro Arg Ala Cys Pro Pro Glu Pro Pro
          20          25          30
Pro Glu His Trp Glu Glu Asp Ala Ser Trp Gly Pro His Arg Leu Ala
          35          40          45
Val Leu Val Pro Phe Arg Glu Arg Phe Glu Glu Leu Leu Val Phe Val
          50          55          60
Pro His Met Arg Arg Phe Leu Ser Arg Lys Lys Ile Arg His His Ile
          65          70          75
Tyr Val Leu Asn Gln Val Asp His Phe Arg Phe Asn Arg Ala Ala Leu
   80           85           90           95
Ile Asn Val Gly Phe Leu Glu Ser Ser Asn Ser Thr Asp Tyr Ile Ala
          100          105          110
Met His Asp Val Asp Leu Leu Pro Leu Asn Glu Glu Leu Asp Tyr Gly
          115          120          125
Phe Pro Glu Ala Gly Pro Phe His Val Ala Ser Pro Glu Leu His Pro
          130          135          140
Leu Tyr His Tyr Lys Thr Tyr Val Gly Gly Ile Leu Leu Leu Ser Lys
          145          150          155
Gln His Tyr Arg Leu Cys Asn Gly Met Ser Asn Arg Phe Trp Gly Trp
   160           165           170           175
Gly Arg Glu Asp Asp Glu Phe Tyr Arg Arg Ile Lys Gly Ala Gly Leu
          180          185          190
Gln Leu Phe Arg Pro Ser Gly Ile Thr Thr Gly Tyr Lys Thr Phe Arg
          195          200          205
His Leu His Asp Pro Ala Trp Arg Lys Arg Asp Gln Lys Arg Ile Ala
          210          215          220
Ala Gln Lys Gln Glu Gln Phe Lys Val Asp Arg Glu Gly Gly Leu Asn
          225          230          235
Thr Val Lys Tyr His Val Ala Ser Arg Thr Ala Leu Ser Val Gly Gly
   240           245           250           255
Ala Pro Cys Thr Val Leu Asn Ile Met Leu Asp Cys Asp Lys Thr Ala
          260          265          270
Thr Pro Trp Cys Thr Phe Ser
          275

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<210> 13  
 <211> 948  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> 80..784

<220>  
 <221> sig\_peptide  
 <222> 80..139  
 <223> Von Heijne matrix  
         score 4  
         seq LLKVVFFVVFASLC/AW

<220>  
 <221> polyA\_signal  
 <222> 910..915

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 933..948

&lt;400&gt; 13

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cttcctgacc caggggctcc gctggctgcg gtcgcctggg agctgccgcc agggccagga      60
ggggagcggc acctggaag atg cgc cca ttg gct ggt ggc ctg ctc aag gtg      112
                Met Arg Pro Leu Ala Gly Gly Leu Leu Lys Val
                -20                -15                -10

gtg ttc gtg gtc ttc gcc tcc ttg tgt gcc tgg tat tgc ggg tac ctg      160
Val Phe Val Val Phe Ala Ser Leu Cys Ala Trp Tyr Ser Gly Tyr Leu
                -5                1                5

ctc gca gag ctc att cca gat gca ccc ctg tcc agt gct gcc tat agc      208
Leu Ala Glu Leu Ile Pro Asp Ala Pro Leu Ser Ser Ala Ala Tyr Ser
                10                15                20

atc cgc agc atc ggg gag agg cct gtc ctc aaa gct cca gtc ccc aaa      256
Ile Arg Ser Ile Gly Glu Arg Pro Val Leu Lys Ala Pro Val Pro Lys
                25                30                35

agg caa aaa tgt gac cac tgg act ccc tgc cca tct gac acc tat gcc      304
Arg Gln Lys Cys Asp His Trp Thr Pro Cys Pro Ser Asp Thr Tyr Ala
                40                45                50                55

tac agg tta ctc agc gga ggt ggc aga agc aag tac gcc aaa atc tgc      352
Tyr Arg Leu Leu Ser Gly Gly Gly Arg Ser Lys Tyr Ala Lys Ile Cys
                60                65                70

ttt gag gat aac cta ctt atg gga gaa cag ctg gga aat gtt gcc aga      400
Phe Glu Asp Asn Leu Leu Met Gly Glu Gln Leu Gly Asn Val Ala Arg
                75                80                85

gga ata aac att gcc att gtc aac tat gta act ggg aat gtg aca gca      448
Gly Ile Asn Ile Ala Ile Val Asn Tyr Val Thr Gly Asn Val Thr Ala
                90                95                100

aca cga tgt ttt gat atg tat gaa ggc gat aac tct gga ccg atg aca      496
Thr Arg Cys Phe Asp Met Tyr Glu Gly Asp Asn Ser Gly Pro Met Thr
                105                110                115

aag ttt att cag agt gct gct cca aaa tcc ctg ctc ttc atg gtg acc      544
Lys Phe Ile Gln Ser Ala Ala Pro Lys Ser Leu Leu Phe Met Val Thr
                120                125                130                135

tat gac gac gga agc aca aga ctg aat aac gat gcc aag aat gcc ata      592
Tyr Asp Asp Gly Ser Thr Arg Leu Asn Asn Asp Ala Lys Asn Ala Ile
                140                145                150

gaa gca ctt gga agt aaa gaa atc agg aac atg aaa ttc agg tct agc      640
Glu Ala Leu Gly Ser Lys Glu Ile Arg Asn Met Lys Phe Arg Ser Ser
                155                160                165

tgg gta ttt att gca gca aaa ggc ttg gaa ctc cct tcc gaa att cag      688
Trp Val Phe Ile Ala Ala Lys Gly Leu Glu Leu Pro Ser Glu Ile Gln
                170                175                180

aga gaa aag atc aac cac tct gat gct aag aac aac aga tat tct ggc      736
Arg Glu Lys Ile Asn His Ser Asp Ala Lys Asn Asn Arg Tyr Ser Gly
                185                190                195

tgg cct gca gag atc cag ata gaa ggc tgc ata ccc aaa gaa cga agc      784
Trp Pro Ala Glu Ile Gln Ile Glu Gly Cys Ile Pro Lys Glu Arg Ser
                200                205                210                215

tgacactgca gggtcctgag taaatgtgtt ctgtataaac aaatgcagct ggaatcgctc      844
aagaatctta tttttctaaa tccaacagcc catatttgat gagtattttg ggtttgttgt      904
aaaccaatga acatttgcta gttgtaccaa aaaaaaaaaa aaaa      948

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&lt;210&gt; 14

&lt;211&gt; 235

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

14

&lt;222&gt; -20...-1

&lt;400&gt; 14

Met Arg Pro Leu Ala Gly Gly Leu Leu Lys Val Val Phe Val Val Phe  
 -20 -15 -10 -5  
 Ala Ser Leu Cys Ala Trp Tyr Ser Gly Tyr Leu Leu Ala Glu Leu Ile  
 1 5 10  
 Pro Asp Ala Pro Leu Ser Ser Ala Ala Tyr Ser Ile Arg Ser Ile Gly  
 15 20 25  
 Glu Arg Pro Val Leu Lys Ala Pro Val Pro Lys Arg Gln Lys Cys Asp  
 30 35 40  
 His Trp Thr Pro Cys Pro Ser Asp Thr Tyr Ala Tyr Arg Leu Leu Ser  
 45 50 55 60  
 Gly Gly Gly Arg Ser Lys Tyr Ala Lys Ile Cys Phe Glu Asp Asn Leu  
 65 70 75  
 Leu Met Gly Glu Gln Leu Gly Asn Val Ala Arg Gly Ile Asn Ile Ala  
 80 85 90  
 Ile Val Asn Tyr Val Thr Gly Asn Val Thr Ala Thr Arg Cys Phe Asp  
 95 100 105  
 Met Tyr Glu Gly Asp Asn Ser Gly Pro Met Thr Lys Phe Ile Gln Ser  
 110 115 120  
 Ala Ala Pro Lys Ser Leu Phe Met Val Thr Tyr Asp Asp Gly Ser  
 125 130 135 140  
 Thr Arg Leu Asn Asn Asp Ala Lys Asn Ala Ile Glu Ala Leu Gly Ser  
 145 150 155  
 Lys Glu Ile Arg Asn Met Lys Phe Arg Ser Ser Trp Val Phe Ile Ala  
 160 165 170  
 Ala Lys Gly Leu Glu Leu Pro Ser Glu Ile Gln Arg Glu Lys Ile Asn  
 175 180 185  
 His Ser Asp Ala Lys Asn Asn Arg Tyr Ser Gly Trp Pro Ala Glu Ile  
 190 195 200  
 Gln Ile Glu Gly Cys Ile Pro Lys Glu Arg Ser  
 205 210 215

&lt;210&gt; 15

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; oligonucleotide used as a primer

&lt;400&gt; 15

gggaagatgg agatagatt gcctg

25

&lt;210&gt; 16

&lt;211&gt; 26

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; oligonucleotide used as a primer

&lt;400&gt; 16

ctgccatgta catgatagag agattc

26

&lt;210&gt; 17

&lt;211&gt; 546

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

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<221> promoter
<222> 1..517

<220>
<221> transcription start site
<222> 518

<220>
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<222> 17..25
<223> matinspector prediction
      name CMYB_01
      score 0.983
      sequence tgtcagttg

<220>
<221> protein_bind
<222> complement(18..27)
<223> matinspector prediction
      name MYOD_Q6
      score 0.961
      sequence cccaactgac

<220>
<221> protein_bind
<222> complement(75..85)
<223> matinspector prediction
      name S8_01
      score 0.960
      sequence aatagaattag

<220>
<221> protein_bind
<222> 94..104
<223> matinspector prediction
      name S8_01
      score 0.966
      sequence aactaaattag

<220>
<221> protein_bind
<222> complement(129..139)
<223> matinspector prediction
      name DELTAEF1_01
      score 0.960
      sequence gcacacctcag

<220>
<221> protein_bind
<222> complement(155..165)
<223> matinspector prediction
      name GATA_C
      score 0.964
      sequence agataaatcca

<220>
<221> protein_bind
<222> 170..178
<223> matinspector prediction
      name CMYB_01
      score 0.958
      sequence cttcagttg
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<220>  
<221> protein\_bind  
<222> 176..189  
<223> matinspector prediction  
name GATA1\_02  
score 0.959  
sequence ttgtagataggaca

<220>  
<221> protein\_bind  
<222> 180..190  
<223> matinspector prediction  
name GATA\_C  
score 0.953  
sequence agataggacat

<220>  
<221> protein\_bind  
<222> 284..299  
<223> matinspector prediction  
name TAL1ALPHA47\_01  
score 0.973  
sequence cataacagatggtaag

<220>  
<221> protein\_bind  
<222> 284..299  
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name TAL1BETA47\_01  
score 0.983  
sequence cataacagatggtaag

<220>  
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<222> 284..299  
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name TAL1BETAITF2\_01  
score 0.978  
sequence cataacagatggtaag

<220>  
<221> protein\_bind  
<222> complement(287..296)  
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name MYOD\_Q6  
score 0.954  
sequence accatctgtt

<220>  
<221> protein\_bind  
<222> complement(302..314)  
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name GATA1\_04  
score 0.953  
sequence tcaagataaagta

<220>  
<221> protein\_bind  
<222> 393..405  
<223> matinspector prediction  
name IK1\_01

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        score 0.963
        sequence agttgggaattcc

<220>
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<222> 393..404
<223> matinspector prediction
        name IK2_01
        score 0.985
        sequence agttgggaattc

<220>
<221> protein_bind
<222> 396..405
<223> matinspector prediction
        name CREL_01
        score 0.962
        sequence tgggaattcc

<220>
<221> protein_bind
<222> 423..436
<223> matinspector prediction
        name GATA1_02
        score 0.950
        sequence tcagtgatatggca

<220>
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<222> complement(478..489)
<223> matinspector prediction
        name SRY_02
        score 0.951
        sequence taaaacaaaaca

<220>
<221> protein_bind
<222> 486..493
<223> matinspector prediction
        name E2F_02
        score 0.957
        sequence tttagcgc

<220>
<221> protein_bind
<222> complement(514..521)
<223> matinspector prediction
        name MZF1_01
        score 0.975
        sequence tgagggga

<400> 17
tgagtgcagt gttacatgtc agttgggtta agtttggttaa tgtcattcaa atcttctatg      60
tcttgatttg cctgctaatt ctattatttc tggaactaaa ttagtttgat ggttctatta      120
gttattgact gaggtgtgct aatctcccat tatgtggatt tatctatttc ttcagttgta      180
gataggacat tgatagatac ataagtacca ggacaaaagc agggagatct tttttccaaa      240
atcaggagaa aaaaatgaca tctggaaaac ctatagggaa aggcataaca gatggtaagg      300
atactttatc ttgagtagga gagccttcct gtggcaacgt ggagaaggga agaggtcgta      360
gaattgagga gtcagctcag ttagaagcag ggagttggga attccgttca tgtgatttag      420
catcagtgat atggcaaatg tgggactaag ggtagtgatc agaggggttaa aattgtgtgt      480
tttgttttag cgctgctggg gcatcgccct gggtccctc aaacagattc ccatgaatct      540
cttcat                                           546

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<210> 18  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> oligonucleotide used as a primer

<400> 18  
gtaccaggga ctgtgacat tgc

23

<210> 19  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> oligonucleotide used as a primer

<400> 19  
ctgtgacat tgctccaag agag

24

<210> 20  
<211> 861  
<212> DNA  
<213> Homo Sapiens

<220>  
<221> promoter  
<222> 1..806

<220>  
<221> transcription start site  
<222> 807

<220>  
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<222> complement(60..70)  
<223> matinspector prediction  
name NFY\_Q6  
score 0.956  
sequence ggaccaatcat

<220>  
<221> protein\_bind  
<222> 70..77  
<223> matinspector prediction  
name MZF1\_01  
score 0.962  
sequence cctgggga

<220>  
<221> protein\_bind  
<222> 124..132  
<223> matinspector prediction  
name CMYB\_01  
score 0.994  
sequence tgaccgttg

<220>  
<221> protein\_bind



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<222> complement(126..134)
<223> matinspector prediction
      name VMYB_02
      score 0.985
      sequence tccaacggt

<220>
<221> protein_bind
<222> 135..143
<223> matinspector prediction
      name STAT_01
      score 0.968
      sequence ttcttgga

<220>
<221> protein_bind
<222> complement(135..143)
<223> matinspector prediction
      name STAT_01
      score 0.951
      sequence ttccaggaa

<220>
<221> protein_bind
<222> complement(252..259)
<223> matinspector prediction
      name MZF1_01
      score 0.956
      sequence ttggggga

<220>
<221> protein_bind
<222> 357..368
<223> matinspector prediction
      name IK2_01
      score 0.965
      sequence gaatgggatttc

<220>
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<222> 384..391
<223> matinspector prediction
      name MZF1_01
      score 0.986
      sequence agagggga

<220>
<221> protein_bind
<222> complement(410..421)
<223> matinspector prediction
      name SRY_02
      score 0.955
      sequence gaaaacaaaaca

<220>
<221> protein_bind
<222> 592..599
<223> matinspector prediction
      name MZF1_01
      score 0.960
      sequence gaagggga
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<220>  
 <221> protein\_bind  
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 <223> matinspector prediction  
       name MYOD\_Q6  
       score 0.981  
       sequence agcatctgcc

<220>  
 <221> protein\_bind  
 <222> 632..642  
 <223> matinspector prediction  
       name DELTAEF1\_01  
       score 0.958  
       sequence tcccaccttcc

<220>  
 <221> protein\_bind  
 <222> complement(813..823)  
 <223> matinspector prediction  
       name S8\_01  
       score 0.992  
       sequence gaggcaattat

<220>  
 <221> protein\_bind  
 <222> complement(824..831)  
 <223> matinspector prediction  
       name MZF1\_01  
       score 0.986  
       sequence agagggga

<220>  
 <221> misc\_feature  
 <222> 335,376  
 <223> n=a, g, c or t

<400> 20  
 tactataggg cacgcgtggt cgacggccgg gctgttcttg agcagagggc atgtcagtaa 60  
 tgattggtcc ctggggaagg tctggctggc tccagcacag tgaggcattt aggtatctct 120  
 cggtgaccgt tggattcctg gaagcagtag ctgttctggt tggatctggg agggacaggg 180  
 ctacagagggc taggcacgag ggaaggtcag aggagaaggs aggsarggcc cagtgaagarg 240  
 ggagcatgcc ttcccccaac cctggcttsc ycttggymam agggcgkty tgggmacttr 300  
 aaytcagggc ccaascagaa scacaggccc aktentggct smaagcacia tagcctgaat 360  
 gggatttcag gttagnacag gtgagagggg aggcctctctg gcttagtttt gttttgtttt 420  
 ccaaatacaag gtaacttgct cccttctgct acgggccttg gtcttggtt gtcttcaccc 480  
 agtcggaact ccctaccact ttcaggagag tggtttttagg cccgtggggc tgttctgttc 540  
 caagcagtggt gagaacatgg ctggttagagg ctctagctgt gtgcggggcc tgaaggggag 600  
 tgggttctcg ccaaagagc atctgcccac ttcccacctt cccttctccc accagaagct 660  
 tgcctgagct gtttggacaa aaatccaaac cccacttggc tactctggcc tggcttcagc 720  
 ttggaaccca atacctaggc ttacaggcca tcctgagcca ggggcctctg gaaattctct 780  
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 tctcttggga gcaatggtca c 861

<210> 21  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> oligonucleotide used as a primer

<400> 21  
ctgggatgga aggcacggta 20

<210> 22  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> oligonucleotide used as a primer

<400> 22  
gagaccacac agctagacaa 20

<210> 23  
<211> 555  
<212> DNA  
<213> Homo Sapiens

<220>  
<221> promoter  
<222> 1..500

<220>  
<221> transcription start site  
<222> 501

<220>  
<221> protein\_bind  
<222> 191..206  
<223> matinspector prediction  
name ARNT\_01  
score 0.964  
sequence ggactcacgtgctgct

<220>  
<221> protein\_bind  
<222> 193..204  
<223> matinspector prediction  
name NMYC\_01  
score 0.965  
sequence actcacgtgctg

<220>  
<221> protein\_bind  
<222> 193..204  
<223> matinspector prediction  
name USF\_01  
score 0.985  
sequence actcacgtgctg

<220>  
<221> protein\_bind  
<222> complement(193..204)  
<223> matinspector prediction  
name USF\_01  
score 0.985  
sequence cagcacgtgagt

<220>  
<221> protein\_bind  
<222> complement(193..204)

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<223> matinspector prediction
      name NMYC_01
      score 0.956
      sequence cagcacgtgagt

<220>
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<222> complement(193..204)
<223> matinspector prediction
      name MYCMAX_02
      score 0.972
      sequence cagcacgtgagt

<220>
<221> protein_bind
<222> 195..202
<223> matinspector prediction
      name USF_C
      score 0.997
      sequence tcacgtgc

<220>
<221> protein_bind
<222> complement(195..202)
<223> matinspector prediction
      name USF_C
      score 0.991
      sequence gcacgtga

<220>
<221> protein_bind
<222> complement(210..217)
<223> matinspector prediction
      name MZF1_01
      score 0.968
      sequence catgggga

<220>
<221> protein_bind
<222> 397..410
<223> matinspector prediction
      name ELK1_02
      score 0.963
      sequence ctctccggaagcct

<220>
<221> protein_bind
<222> 400..409
<223> matinspector prediction
      name CETS1P54_01
      score 0.974
      sequence tccggaagcc

<220>
<221> protein_bind
<222> complement(460..470)
<223> matinspector prediction
      name AP1_Q4
      score 0.963
      sequence agtgactgaac

<220>
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<221> protein\_bind  
 <222> complement(460..470)  
 <223> matinspector prediction  
       name AP1FJ\_Q2  
       score 0.961  
       sequence agtgactgaac

<220>  
 <221> protein\_bind  
 <222> 547..555  
 <223> matinspector prediction  
       name PADS\_C  
       score 1.000  
       sequence tgtggtctc

<400> 23  
 ctatagggca cgcktggtcg acggcccggg ctggtctggt ctgktgtgga gtcggggtga 60  
 aggacagcat ttgtkacatc tggctactg caccttccct ctgccgtgca cttggccttt 120  
 kawaagctca gcaccggtgc ccatcacagg gccggcagca cacacatccc attactcaga 180  
 aggaactgac ggactcacgt gctgctccgt ccccatgagc tcagtggacc tgtctatgta 240  
 gagcagtcag acagtgcctg ggatagagtg agagttcagc cagtaaatcc aagtgattgt 300  
 cattcctgtc tgcattagta actcccaacc tagatgtgaa aacttagttc tttctcatag 360  
 gttgctctgc ccattggtccc actgcagacc caggcactct ccggaagcct ggaaatcacc 420  
 cgtgtcttct gcctgctccc gctcacatcc cacacttgtg ttcagtcact gagttacaga 480  
 ttttgctccc tcaatttctc ttgtcttagt cccatcctct gttcccctgg ccagtttgtc 540  
 tagctgtgtg gtctc 555

<210> 24  
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 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 153..1127

<220>  
 <221> sig\_peptide  
 <222> 153..230  
 <223> Von Heijne matrix  
       score 8.40  
       seq RLLRLLLSGLVLG/AA

<220>  
 <221> polyA\_signal  
 <222> 1415..1420

<220>  
 <221> polyA\_site  
 <222> 1434..1450

<220>  
 <221> misc\_feature  
 <222> 88  
 <223> n=a, g, c or t

<400> 24  
 ctttctcttt cctctcctc ctcttgga tccgctctt ctctctctg cgtctctccc 60  
 cgctgctccc gctgctccc acgcggancc cggagcccgc gccgagcccc tggcctcgcg 120  
 gtgccatgct gcccggcg cggcgtgaa gg atg gcg acg ccg ctg cct ccg 173  
                   Met Ala Thr Pro Leu Pro Pro  
                   -25 -20

ccc tcc ccg cgg cac ctg cgg ctg ctg cgg ctg ctg ctc tcc ggc ctc	221
Pro Ser Pro Arg His Leu Arg Leu Leu Arg Leu Leu Leu Ser Gly Leu	
-15 -10 -5	
gtc ctc ggc gcc gcc ctg cgt gga gcc gcc gcc ggc cac ccg gat gta	269
Val Leu Gly Ala Ala Leu Arg Gly Ala Ala Ala Gly His Pro Asp Val	
1 5 10	
gcc gcc tgt ccc ggg agc ctg gac tgt gcc ctg aag agg cgg gca agg	317
Ala Ala Cys Pro Gly Ser Leu Asp Cys Ala Leu Lys Arg Arg Ala Arg	
15 20 25	
tgt cct cct ggt gca cat gcc tgt ggg ccc tgc ctt cag ccc ttc cag	365
Cys Pro Pro Gly Ala His Ala Cys Gly Pro Cys Leu Gln Pro Phe Gln	
30 35 40 45	
gag gac cag caa ggg ctc tgt gtg ccc agg atg cgc cgg cct cca ggc	413
Glu Asp Gln Gln Leu Cys Val Pro Arg Met Arg Arg Pro Pro Gly	
50 55 60	
ggg ggc cgg ccc cag ccc aga ctg gaa gat gag att gac ttc ctg gcc	461
Gly Gly Arg Pro Gln Pro Arg Leu Glu Asp Glu Ile Asp Phe Leu Ala	
65 70 75	
cag gag ctt gcc cgg aag gag tct gga cac tca act ccg ccc cta ccc	509
Gln Glu Leu Ala Arg Lys Glu Ser Gly His Ser Thr Pro Pro Leu Pro	
80 85 90	
aag gac cga cag cgg ctc ccg gag cct gcc acc ctg ggc ttc tcg gca	557
Lys Asp Arg Gln Arg Leu Pro Glu Pro Ala Thr Leu Gly Phe Ser Ala	
95 100 105	
cgg ggg cag ggg ctg gag ctg ggc ctc ccc tcc act cca gga acc ccc	605
Arg Gly Gln Gly Leu Glu Leu Gly Leu Pro Ser Thr Pro Gly Thr Pro	
110 115 120 125	
acg ccc acg ccc cac acc tcc ctg ggc tcc cct gtg tca tcc gac ccg	653
Thr Pro Thr Pro His Thr Ser Leu Gly Ser Pro Val Ser Ser Asp Pro	
130 135 140	
gtg cac atg tcg ccc ctg gag ccc cgg gga ggg caa ggc gac ggc ctc	701
Val His Met Ser Pro Leu Glu Pro Arg Gly Gly Gln Gly Asp Gly Leu	
145 150 155	
gcc ctt gtg ctg atc ctg gcg ttc tgt gtg gcc ggt gca gcc gcc ctc	749
Ala Leu Val Leu Ile Leu Ala Phe Cys Val Ala Gly Ala Ala Ala Leu	
160 165 170	
tcc gta gcc tcc ctc tgc tgg tgc agg ctg cag cgt gag atc cgc ctg	797
Ser Val Ala Ser Leu Cys Trp Cys Arg Leu Gln Arg Glu Ile Arg Leu	
175 180 185	
act cag aag gcc gac tac gcc act gcg aag gcc cct ggc tca cct gca	845
Thr Gln Lys Ala Asp Tyr Ala Thr Ala Lys Ala Pro Gly Ser Pro Ala	
190 195 200 205	
gct ccc cgg atc tcg cct ggg gac cag cgg ctg gca cag agc gcg gag	893
Ala Pro Arg Ile Ser Pro Gly Asp Gln Arg Leu Ala Gln Ser Ala Glu	
210 215 220	
atg tac cac tac cag cac caa cgg caa cag atg ctg tgc ctg gag cgg	941
Met Tyr His Tyr Gln His Gln Arg Gln Gln Met Leu Cys Leu Glu Arg	
225 230 235	
cat aaa gag cca ccc aag gag ctg gac acg gcc tcc tcg gat gag gag	989
His Lys Glu Pro Pro Lys Glu Leu Asp Thr Ala Ser Ser Asp Glu Glu	
240 245 250	
aat gag gac gga gac ttc acg gtg tac gag tgc ccg ggc ctg gcc ccg	1037
Asn Glu Asp Gly Asp Phe Thr Val Tyr Glu Cys Pro Gly Leu Ala Pro	
255 260 265	
acc ggg gaa atg gag gtg cgc aac cct ctg ttc gac cac gcc gca ctg	1085
Thr Gly Glu Met Glu Val Arg Asn Pro Leu Phe Asp His Ala Ala Leu	
270 275 280 285	
tcc gcg ccc ctg ccg gcc ccc agc tca ccg cct gca ctg cca	1127
Ser Ala Pro Leu Pro Ala Pro Ser Ser Pro Pro Ala Leu Pro	
290 295	
tgacctggag gcagacagac gccacactgc tccccgacct cgaggccccc ggggaggggc	1187
agggcctgga gcttcccact aaaaacatgt tttgatgctg tgtgcttttg gctgggcctt	1247

25

```

gggctccagg ccctgggacc ccttgccagg gagacccccg aacctttgtg ccaggacacc 1307
tcctgggtccc ctgcacctct cctgttttgg ttagaccccc aaactggagg gggcatggag 1367
aaccgtagag cgcaggaacg ggtgggtaat tctagagaca aaagccaatt aaagtccatt 1427
tcagacaaaa aaaaaaaaaa aaa 1450

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<210> 25  
 <211> 1556  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 261..1166

<220>  
 <221> sig\_peptide  
 <222> 261..314  
 <223> Von Heijne matrix  
         score 8.80  
         seq RLVLIIILCSVVFS/AV

<220>  
 <221> polyA\_site  
 <222> 1524..1556

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<400> 25
cagcccagtc ggcccggccc gggggccatg gagctccgag cggcgatcgc gaggctctctg 60
cgaacccag cctgcacgcc cggtagcat tcggccggga gatgcggcag tggaatctgg 120
aagggcggtg aaaaacctac gtccctgccct cgcccgccct ctccattcgt ccccgggta 180
gagaggtgcc cggtcccac cccttcccag cccagccct ggagacagca gcccctagac 240
tactgagggga cagcgacagc atg aag gct ccg ggt cgg ctc gtg ctc atc atc 293
                        Met Lys Ala Pro Gly Arg Leu Val Leu Ile Ile
                        -15                               -10

ctg tgc tcc gtg gtc ttc tct gcc gtc tac atc ctc ctg tgc tgc tgg 341
Leu Cys Ser Val Val Phe Ser Ala Val Tyr Ile Leu Leu Cys Cys Trp
                        -5                               1                               5
gcc ggc ctg ccc ctc tgc ctg gcc acc tgc ctg gac cac cac ttc ccc 389
Ala Gly Leu Pro Leu Cys Leu Ala Thr Cys Leu Asp His His Phe Pro
10                               15                               20                               25
aca ggc tcc agg ccc act gtg ccg gga ccc ctg cac ttc agt gga tat 437
Thr Gly Ser Arg Pro Thr Val Pro Gly Pro Leu His Phe Ser Gly Tyr
                        30                               35                               40
agc agt gtg cca gat ggg aag ccg ctg gtc cgc gag ccc tgc cgc agc 485
Ser Ser Val Pro Asp Gly Lys Pro Leu Val Arg Glu Pro Cys Arg Ser
                        45                               50                               55
tgt gcc gtg gtg tcc agc tcc ggc caa atg ctg ggc tca ggc ctg ggt 533
Cys Ala Val Val Ser Ser Ser Gly Gln Met Leu Gly Ser Gly Leu Gly
60                               65                               70
gct gag atc gac agt gcc gag tgc gtg ttc cgc atg aac cag gcg ccc 581
Ala Glu Ile Asp Ser Ala Glu Cys Val Phe Arg Met Asn Gln Ala Pro
75                               80                               85
acc gtg ggc ttt gag gcg gat gtg ggc cag cgc agc acc ctg cgt gtc 629
Thr Val Gly Phe Glu Ala Asp Val Gly Gln Arg Ser Thr Leu Arg Val
90                               95                               100                               105
gtc tca cac aca agc gtg ccg ctg ctg ctg cgc aac tat tca cac tac 677
Val Ser His Thr Ser Val Pro Leu Leu Leu Arg Asn Tyr Ser His Tyr
110                               115                               120
ttc cag aag gcc cga gac acg ctc tac atg gtg tgg ggc cag ggc agg 725
Phe Gln Lys Ala Arg Asp Thr Leu Tyr Met Val Trp Gly Gln Gly Arg
125                               130                               135
cac atg gac cgg gtg ctc ggc ggc cgc acc tac cgc acg ctg cag 773
His Met Asp Arg Val Leu Gly Gly Arg Thr Tyr Arg Thr Leu Leu Gln

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26

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      140      145      150
ctc acc agg atg tac ccc ggc ctg cag gtg tac acc ttc acg gag cgc      821
Leu Thr Arg Met Tyr Pro Gly Leu Gln Val Tyr Thr Phe Thr Glu Arg
      155      160      165
atg atg gcc tac tgc gac cag atc ttc cag gac gag acg ggc aag aac      869
Met Met Ala Tyr Cys Asp Gln Ile Phe Gln Asp Glu Thr Gly Lys Asn
      170      175      180      185
cgg agg cag tgc ggc tcc ttc ctc agc acc ggc tgg ttc acc atg atc      917
Arg Arg Gln Ser Gly Ser Phe Leu Ser Thr Gly Trp Phe Thr Met Ile
      190      195      200
ctc gcg ctg gag ctg tgt gag gag atc gtg gtc tat ggg atg gtc agc      965
Leu Ala Leu Glu Leu Cys Glu Glu Ile Val Val Tyr Gly Met Val Ser
      205      210      215
gac agc tac tgc agg gag aag agc cac ccc tca gtg cct tac cac tac      1013
Asp Ser Tyr Cys Arg Glu Lys Ser His Pro Ser Val Pro Tyr His Tyr
      220      225      230
ttt gag aag ggc cgg cta gat gag tgt cag atg tac ctg gca cac gag      1061
Phe Glu Lys Gly Arg Leu Asp Glu Cys Gln Met Tyr Leu Ala His Glu
      235      240      245
cag gcg ccc cga agc gcc cac cgc ttc atc act gag aag gcg gtc ttc      1109
Gln Ala Pro Arg Ser Ala His Arg Phe Ile Thr Glu Lys Ala Val Phe
      250      255      260      265
tcc cgc tgg gcc aag aag agg ccc atc gtg ttc gcc cat ccg tcc tgg      1157
Ser Arg Trp Ala Lys Lys Arg Pro Ile Val Phe Ala His Pro Ser Trp
      270      275      280
agg act gag tagcttccgt cgtcctgccca gccgccatgc cgttgcgagg      1206
Arg Thr Glu
cctccgggat gtcccatccc aagccatcac actccactcc ctgagtaatt catggcattt      1266
ggggggtcac cacctccagg tctgtcaagt ggcctttgtc cctgggggtg atggccccc      1326
actcaccagc atcatgacct tgtgccagtc ctggtcctcc ctcccagcc gccctacca      1386
ccttttgggtg ccacacttct caggctggcc gccctgggtg gggcagccga gagcctgggg      1446
ttcattgggtg aaggggcctt ggagttgtga ctgccggggc cgtatcagga acgtacgggt      1506
aaacgtgtgt tttctggaaa aaaaaaaaaa aacaaaaaaaaa aaaaaaaaaa      1556

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<210> 26  
 <211> 1058  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 67..813

<220>  
 <221> sig\_peptide  
 <222> 67..111  
 <223> Von Heijne matrix  
 score 5.20  
 seq QLWKLVLCCGVL/GT

<220>  
 <221> polyA\_signal  
 <222> 1023..1028

<220>  
 <221> polyA\_site  
 <222> 1042..1058

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<400> 26
agcagactgt gcagtggggc aaggatttca tgagcatcct cctctaaacg cgtgacaaga      60
caaaag atg ctt cag ctt tgg aaa ctt gtt ctc ctg tgc ggc gtg ctc      108
      Met Leu Gln Leu Trp Lys Leu Val Leu Leu Cys Gly Val Leu

```



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-15          -10          -5
act ggg acc tca gag tct ctt ctt gac aat ctt ggc aat gac cta agc      156
Thr Gly Thr Ser Glu Ser Leu Leu Asp Asn Leu Gly Asn Asp Leu Ser
1          5          10          15
aat gtc gtg gat aag ctg gaa cct gtt ctt cac gag gga ctt gag aca      204
Asn Val Val Asp Lys Leu Glu Pro Val Leu His Glu Gly Leu Glu Thr
20          25          30
gtt gac aat act ctt aaa ggc atc ctt gag aaa ctg aag gtc gac cta      252
Val Asp Asn Thr Leu Lys Gly Ile Leu Glu Lys Leu Lys Val Asp Leu
35          40          45
gga gtg ctt cag aaa tcc agt gct tgg caa ctg gcc aag cag aag gcc      300
Gly Val Leu Gln Lys Ser Ser Ala Trp Gln Leu Ala Lys Gln Lys Ala
50          55          60
cag gaa gct gag aaa ttg ctg aac aat gtc att tct aag ctg ctt cca      348
Gln Glu Ala Glu Lys Leu Leu Asn Asn Val Ile Ser Lys Leu Leu Pro
65          70          75
act aac acg gac att ttt ggg ttg aaa atc agc aac tcc ctc atc ctg      396
Thr Asn Thr Asp Ile Phe Gly Leu Lys Ile Ser Asn Ser Leu Ile Leu
80          85          90          95
gat gtc aaa gct gaa ccg atc gat gat ggc aaa ggc ctt aac ctg agc      444
Asp Val Lys Ala Glu Pro Ile Asp Asp Gly Lys Gly Leu Asn Leu Ser
100          105          110
ttc cct gtc acc gcg aat gtc act gtg gcc ggg ccc atc att ggc cag      492
Phe Pro Val Thr Ala Asn Val Thr Val Ala Gly Pro Ile Ile Gly Gln
115          120          125
att atc aac ctg aaa gcc tcc ttg gac ctc ctg acc gca gtc aca att      540
Ile Ile Asn Leu Lys Ala Ser Leu Asp Leu Leu Thr Ala Val Thr Ile
130          135          140
gaa act gat ccc cag aca cac cag cct gtt gcc gtc ctg gga gaa tgc      588
Glu Thr Asp Pro Gln Thr His Gln Pro Val Ala Val Leu Gly Glu Cys
145          150          155
gcc agt gac cca acc agc atc tca ctt tcc ttg ctg gac aaa cac agc      636
Ala Ser Asp Pro Thr Ser Ile Ser Leu Ser Leu Leu Asp Lys His Ser
160          165          170          175
caa atc atc aac aag ttc gtg aat agc gtg atc aac acg ctg aaa agc      684
Gln Ile Ile Asn Lys Phe Val Asn Ser Val Ile Asn Thr Leu Lys Ser
180          185          190
act gta tcc tcc ctg ctg cag aag gag ata tgt cca ctg atc cgc atc      732
Thr Val Ser Ser Leu Leu Gln Lys Glu Ile Cys Pro Leu Ile Arg Ile
195          200          205
ttc atc cac tcc ctg gat gtg aat gtc att cag cag gtc gtc gat aat      780
Phe Ile His Ser Leu Asp Val Asn Val Ile Gln Gln Val Val Asp Asn
210          215          220
cct cag cac aaa acc cag ctg caa acc ctc atc tgaagaggac gaatgaggag      833
Pro Gln His Lys Thr Gln Leu Gln Thr Leu Ile
225          230
gaccactgtg gtgcatgctg attggttccc agtggcttgc cccacccct tatagcatct      893
ccctccagga agctgctgcc accacctaac cagcgtgaaa gcctgagtcc caccagaagg      953
accttcccag ataccccttc tcctcacagt cagaacagca gcctctacac atgttgctct      1013
gcccctggca ataaaggccc atttctgcaa aaaaaaaaaa aaaaa      1058

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&lt;210&gt; 27

&lt;211&gt; 648

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 187..438

&lt;220&gt;

&lt;221&gt; polyA\_signal

<222> 612..617

<220>

<221> polyA\_site

<222> 632..648

<400> 27

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agtgcgcact ggcgtgag actcggcggg cgctgttgag ggagtcgggc cgcgactgtg      60
gtcgttttta taccttcccg cgcggacgcc ggcgttgcca acggaagggc ggagacggag      120
tttcgtcatg ttggccaggc ccatttgaga tctttgaaga taccctcaac gtgaggctct      180
gctgcc atg aag gtg aag att aag tgc tgg aac ggc gtg gcc act tgg      228
      Met Lys Val Lys Ile Lys Cys Trp Asn Gly Val Ala Thr Trp
          1             5             10
ctc tgg gtg gcc aac gat gag aac tgt ggc atc tgc agg atg gca ttt      276
Leu Trp Val Ala Asn Asp Glu Asn Cys Gly Ile Cys Arg Met Ala Phe
15             20             25             30
aac gga tgc tgc cct gac tgc aag gtg ccc ggc gac gac tgc ccg ctg      324
Asn Gly Cys Cys Pro Asp Cys Lys Val Pro Gly Asp Asp Cys Pro Leu
          35             40             45
gtg tgg ggc cag tgc tcc cac tgc ttc cac atg cat tgc atc ctc aag      372
Val Trp Gly Gln Cys Ser His Cys Phe His Met His Cys Ile Leu Lys
          50             55             60
tgg ctg cac gca cag cag gtg cag cag cac tgc ccc atg tgc cgc cag      420
Trp Leu His Ala Gln Gln Val Gln Gln His Cys Pro Met Cys Arg Gln
          65             70             75
gaa tgg aag ttc aag gag tgaggcccg cctggctctc gctggagggg      468
Glu Trp Lys Phe Lys Glu
          80
catcctgaga ctccttcctc atgctggcgc cgatggctgc tggggacagc gcccctgagc      528
tgcaacaagg tggaaacaag ggctggagct gcgtttgttt tgccatcact atgttgacac      588
ttttatccaa taagtgaata ctcattaaac tactcaaata tcgaaaaaaaa aaaaaaaaaa      648

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<210> 28

<211> 2104

<212> DNA

<213> Homo Sapiens

<220>

<221> CDS

<222> 92..1753

<220>

<221> sig\_peptide

<222> 92..130

<223> Von Heijne matrix

score 3.90

seq MLYLQGWSMPAVA/EV

<220>

<221> polyA\_signal

<222> 2070..2075

<220>

<221> polyA\_site

<222> 2090..2104

<220>

<221> misc\_feature

<222> 905

<223> n=a, g, c or t

<220>

&lt;221&gt; unsure

&lt;222&gt; 259

&lt;223&gt; Xaa = Asp, His, Asn, Tyr

&lt;400&gt; 28

```

atagaatttta tcataacttcg tagcatccag tatgttttct ttgctaagat tattgatttt 60
gtattgaagg gtcccatgtc catcgttttc a atg ctt tat ctc cag ggt tgg 112
                                Met Leu Tyr Leu Gln Gly Trp
                                -10
agc atg cct gct gtg gca gag gta aaa ctt cga gat gat caa tat aca 160
Ser Met Pro Ala Val Ala Glu Val Lys Leu Arg Asp Asp Gln Tyr Thr
-5 1 5 10
ctg gaa cac atg cat gct ttt gga atg tat aat tac ctg cac tgt gat 208
Leu Glu His Met His Ala Phe Gly Met Tyr Asn Tyr Leu His Cys Asp
15 20 25
tca tgg tat caa gac agt gtc tac tat att gat acc ctt gga aga att 256
Ser Trp Tyr Gln Asp Ser Val Tyr Tyr Ile Asp Thr Leu Gly Arg Ile
30 35 40
atg aat tta aca gta atg ctg gac act gcc tta gga aaa cca cga gag 304
Met Asn Leu Thr Val Met Leu Asp Thr Ala Leu Gly Lys Pro Arg Glu
45 50 55
gtg ttt cga ctt cct aca gat ttg aca gca tgt gac aac cgt ctt tgt 352
Val Phe Arg Leu Pro Thr Asp Leu Thr Ala Cys Asp Asn Arg Leu Cys
60 65 70
gca tct atc cat ttc tca tct tct acc tgg gtt acc ttg tca gat gga 400
Ala Ser Ile His Phe Ser Ser Ser Thr Trp Val Thr Leu Ser Asp Gly
75 80 85 90
act gga aga ttg tat gtc att gga aca ggt gaa cgt gga aat agc gct 448
Thr Gly Arg Leu Tyr Val Ile Gly Thr Gly Glu Arg Gly Asn Ser Ala
95 100 105
tct gaa aaa tgg gag att atg ttt aat gaa gaa ctt ggg gat cct ttt 496
Ser Glu Lys Trp Glu Ile Met Phe Asn Glu Glu Leu Gly Asp Pro Phe
110 115 120
att ata att cac agt atc tca ctg cta aat gct gaa gaa cat tct ata 544
Ile Ile Ile His Ser Ile Ser Leu Leu Asn Ala Glu Glu His Ser Ile
125 130 135
gct acc cta ctt ctt cga ata gag aaa gag gaa ttg gat atg aaa gga 592
Ala Thr Leu Leu Leu Arg Ile Glu Lys Glu Glu Leu Asp Met Lys Gly
140 145 150
agt ggt ttc tat gtt tct ctg gag tgg gtc act atc agt aag aaa aat 640
Ser Gly Phe Tyr Val Ser Leu Glu Trp Val Thr Ile Ser Lys Lys Asn
155 160 165 170
caa gat aat aaa aaa tat gaa att att aag cgt gat att ctc cgt gga 688
Gln Asp Asn Lys Lys Tyr Glu Ile Ile Lys Arg Asp Ile Leu Arg Gly
175 180 185
aag tca gtg cca cat tat gct gct att aag cct gat gga aat ggt cta 736
Lys Ser Val Pro His Tyr Ala Ala Ile Lys Pro Asp Gly Asn Gly Leu
190 195 200
atg att gta tcc tac aag tct tta aca ttt gtt cag gct ggt caa gat 784
Met Ile Val Ser Tyr Lys Ser Leu Thr Phe Val Gln Ala Gly Gln Asp
205 210 215
ctt gaa gaa aat atg gat gaa gac ata tca gag aaa atc aaa gaa cct 832
Leu Glu Glu Asn Met Asp Glu Asp Ile Ser Glu Lys Ile Lys Glu Pro
220 225 230
ctg tat tac tgg caa cag act gaa gat gat ttg aca gta acc ata cgg 880
Leu Tyr Tyr Trp Gln Gln Thr Glu Asp Asp Leu Thr Val Thr Ile Arg
235 240 245 250
ctt cca gaa gac agt act aag gag nac att caa ata cag ttt ttg cct 928
Leu Pro Glu Asp Ser Thr Lys Glu Xaa Ile Gln Ile Gln Phe Leu Pro
255 260 265
gat cac atc aac att gta ctg aag gat cac cag ttt tta gaa gga aaa 976
Asp His Ile Asn Ile Val Leu Lys Asp His Gln Phe Leu Glu Gly Lys

```

```

      270      275      280
ctc tat tca tct att gat cat gaa agc agt aca tgg ata att aaa gag      1024
Leu Tyr Ser Ser Ile Asp His Glu Ser Ser Thr Trp Ile Ile Lys Glu
      285      290      295
agt aat agc ttg gag att tcc ttg att aag aag aat gaa gga ctg acc      1072
Ser Asn Ser Leu Glu Ile Ser Leu Ile Lys Lys Asn Glu Gly Leu Thr
      300      305      310
tgg cca gag cta gta att gga gat aaa caa ggg gaa ctt ata aga gat      1120
Trp Pro Glu Leu Val Ile Gly Asp Lys Gln Gly Glu Leu Ile Arg Asp
      315      320      325      330
tca gcc cag tgt gct gca ata gct gaa cgt ttg atg cat ttg acc tct      1168
Ser Ala Gln Cys Ala Ala Ile Ala Glu Arg Leu Met His Leu Thr Ser
      335      340      345
gaa gaa ctg aat cca aat cca gat aaa gaa aaa cca cct tgc aat gct      1216
Glu Glu Leu Asn Pro Asn Pro Asp Lys Glu Lys Pro Pro Cys Asn Ala
      350      355      360
caa gag tta gaa gaa tgt gat att ttc ttt gaa gag agc tcc agt tta      1264
Gln Glu Leu Glu Glu Cys Asp Ile Phe Phe Glu Glu Ser Ser Ser Leu
      365      370      375
tgc aga ttt gat ggc aat aca tta aaa act act cat gtg gtg aat ctt      1312
Cys Arg Phe Asp Gly Asn Thr Leu Lys Thr Thr His Val Val Asn Leu
      380      385      390
gga agc aac cag tac ctt ttc tct gtc ata gtg gat cct aaa gaa atg      1360
Gly Ser Asn Gln Tyr Leu Phe Ser Val Ile Val Asp Pro Lys Glu Met
      395      400      405      410
ccc tgc ttc tgt ttg cgc cat gat gtt gat gcc cta ctc tgg caa cca      1408
Pro Cys Phe Cys Leu Arg His Asp Val Asp Ala Leu Leu Trp Gln Pro
      415      420      425
cac tcc agc aaa caa gat gat atg tgg gag cac atc gca act ttc aat      1456
His Ser Ser Lys Gln Asp Asp Met Trp Glu His Ile Ala Thr Phe Asn
      430      435      440
gct tta ggc tat gtc caa gca tca aag aga gac aaa aaa ttt ttt gcc      1504
Ala Leu Gly Tyr Val Gln Ala Ser Lys Arg Asp Lys Lys Phe Phe Ala
      445      450      455
tgt gct cca aat tac tcg tat gca gcc ctt tgt gag tgc ctt cgt cga      1552
Cys Ala Pro Asn Tyr Ser Tyr Ala Ala Leu Cys Glu Cys Leu Arg Arg
      460      465      470
gta ttc atc tat cgt cag cct gct ccc atg tcc act gta ctt tac aac      1600
Val Phe Ile Tyr Arg Gln Pro Ala Pro Met Ser Thr Val Leu Tyr Asn
      475      480      485      490
aga aag gaa ggc agg caa gta gga cag gtt gct aag cag caa gta gca      1648
Arg Lys Glu Gly Arg Gln Val Gly Gln Val Ala Lys Gln Gln Val Ala
      495      500      505
agc cta gaa acc aat gat cct att tta gga ttt cag gca aca aat gag      1696
Ser Leu Glu Thr Asn Asp Pro Ile Leu Gly Phe Gln Ala Thr Asn Glu
      510      515      520
aga tta ttt gtt ctt act acc aaa aac ctc ttt tta ata aaa gta aat      1744
Arg Leu Phe Val Leu Thr Thr Lys Asn Leu Phe Leu Ile Lys Val Asn
      525      530      535
aca gag aat taattattct aacatattgg cctctttgta ctggaaaagt      1793
Thr Glu Asn
      540
attcagtggt acctggaggt ctggacagtt atactgtaac ctcttaagtt ttaatgtgct      1853
aaatatatct tgtatgattt tttatttttt aataacattg gaaatatatt caagagatta      1913
tgattctgta aagctgtgga atgaagctgc agatttagag aacattggct tctgaaaaaa      1973
aaaaagagtg aagatagtag tagcaagtat acttattttt taaaacaggc tagaatctca      2033
tgttttatat gaaagatgta caattcagtg tttaaaaata aaaatattta ttgtgtaaaa      2093
aaaaaaaaa a      2104

```

&lt;210&gt; 29

&lt;211&gt; 515

&lt;212&gt; DNA

31

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 144..440

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 144..287

&lt;223&gt; Von Heijne matrix

score 4.10

seq VFMLIVSVLALIP/ET

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 457..462

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 500..515

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 60

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 29

```

agagagcggg aagccgagct gggcgagaag taggggaggg cgggtgctccg cgcggtggcn      60
gttgctatcg cttcgagaa cctactcagg cagccagctg agaagagttg agggaaagtg      120
ctgctgctgg gtctgcagac gcg atg gat aac gtg cag ccg aaa ata aaa cat      173
                               Met Asp Asn Val Gln Pro Lys Ile Lys His
                               -45                               -40

cgc ccc ttc tgc ttc agt gtg aaa ggc cac gtg aag atg ctg cgg ctg      221
Arg Pro Phe Cys Phe Ser Val Lys Gly His Val Lys Met Leu Arg Leu
                               -35                               -30                               -25

gat att atc aac tca ctg gta aca aca gta ttc atg ctc atc gta tct      269
Asp Ile Ile Asn Ser Leu Val Thr Thr Val Phe Met Leu Ile Val Ser
                               -20                               -15                               -10

gtg ttg gca ctg ata cca gaa acc aca aca ttg aca gtt ggt gga ggg      317
Val Leu Ala Leu Ile Pro Glu Thr Thr Thr Leu Thr Val Gly Gly Gly
                               -5                               1                               5                               10

gtg ttt gca ctt gtg aca gca gta tgc tgt ctt gcc gac ggg gcc ctt      365
Val Phe Ala Leu Val Thr Ala Val Cys Cys Leu Ala Asp Gly Ala Leu
                               15                               20                               25

att tac cgg aag ctt ctg ttc aat ccc agc ggt cct tac cag aaa aag      413
Ile Tyr Arg Lys Leu Leu Phe Asn Pro Ser Gly Pro Tyr Gln Lys Lys
                               30                               35                               40

cct gtg cat gaa aaa aaa gaa gtt ttg taattttata ttacttttta      460
Pro Val His Glu Lys Lys Glu Val Leu
                               45                               50

gtttgatact aagtattaaa catatttctg tattcttcca aaaaaaaaaa aaaat      515

```

&lt;210&gt; 30

&lt;211&gt; 661

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 174..443

&lt;220&gt;

```
<221> sig_peptide
<222> 174..269
<223> Von Heijne matrix
      score 4.10
      seq SSLAFQVGFLLTA/QP
```

```
<220>  
<221> polyA_signal  
<222> 623..628
```

```
<220>
<221> polyA_site
<222> 647..661
```

[illegible]

```
<210> 31
<211> 694
<212> DNA
<213> Homo Sapiens
```

```
<220>  
<221> CDS  
<222> 55..399
```

```
<220>
<221> sig_peptide
<222> 55..192
<223> Von Heijne matrix
      score 4.70
      seq ILTGLTVGSAADA/GE
```

```
<220>
<221> polyA_signal
<222> 654..659
```

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 680..694

&lt;400&gt; 31

```

aatgcttgag gaaaactggg aacagtatat tggtctgaaa acctaaaaag ttta atg      57
                                         Met
aaa acc ttg ttc aat cca gcc cct gcc att gct gac ctg gat ccc cag      105
Lys Thr Leu Phe Asn Pro Ala Pro Ala Ile Ala Asp Leu Asp Pro Gln
-45                               -40                               -35                               -30
ttc tac acc ctc tca gat gtg ttc tgc tgc aat gaa agt gag gct gag      153
Phe Tyr Thr Leu Ser Asp Val Phe Cys Cys Asn Glu Ser Glu Ala Glu
                               -25                               -20                               -15
att tta act ggc ctc acg gtg ggc agc gct gca gat gct ggg gag gct      201
Ile Leu Thr Gly Leu Thr Val Gly Ser Ala Ala Asp Ala Gly Glu Ala
                               -10                               -5                               1
gca tta gtg ctc ttg aaa agg ggc tgc cag gtg gta atc att acc tta      249
Ala Leu Val Leu Leu Lys Arg Gly Cys Gln Val Val Ile Ile Thr Leu
5                               10                               15
ggg gct gaa gga tgt gtg gtg ctg tca cag aca gaa cct gag cca aag      297
Gly Ala Glu Gly Cys Val Val Leu Ser Gln Thr Glu Pro Glu Pro Lys
20                               25                               30                               35
cac att ccc aca gag aaa gtc aag gct gtg gat acc acg tgt aga cct      345
His Ile Pro Thr Glu Lys Val Lys Ala Val Asp Thr Thr Cys Arg Pro
                               40                               45                               50
ggc tca aga ccc aag agt gaa gca gca agt gtg aag aag cag aaa cat      393
Gly Ser Arg Pro Lys Ser Glu Ala Ala Ser Val Lys Lys Gln Lys His
                               55                               60                               65
tat aaa taaccagag aatcctttta taacagcaac tgcctactga ttttgtggcc      449
Tyr Lys
taacagctcg agcaaaaatg aatataaata caacattgtg caatgactaa ttactcaaaa      509
ttttgtgcat cagcagaagt ggaacctgtg gttggtgcta atattatgaa atgcctttgc      569
tgtttaataa tctggtagct ctgtattatt tagcatgcat ttttcttggg gaacaatgat      629
tttatttcaa gtacctctca ctgaaataaa aaagcagctg ttagaagacg aaaaaaaaaa      689
aaaaa                                                                694

```

&lt;210&gt; 32

&lt;211&gt; 1110

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 90..287

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 90..146

&lt;223&gt; Von Heijne matrix

score 9.30

seq VFVFLFLWDPVLA/GI

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1078..1083

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1096..1110

&lt;400&gt; 32

```

atcatcttac atcagcacia gaagaagagt gagcatagca caccgatgtc agaccctgcc      60

```

34

```

actagcctcc ttaacagaag ttcccagcc atg aag cct ctc ctt gtt gtg ttt      113
                                Met Lys Pro Leu Leu Val Val Phe
                                -15
gtc ttt ctt ttc ctt tgg gat cca gtg ctg gca ggt ata aat tca tta      161
Val Phe Leu Phe Leu Trp Asp Pro Val Leu Ala Gly Ile Asn Ser Leu
-10      -5      1      5
tca tca gaa atg cac aag aaa tgc tat aaa aat ggc atc tgc aga ctt      209
Ser Ser Glu Met His Lys Lys Cys Tyr Lys Asn Gly Ile Cys Arg Leu
      10      15      20
gaa tgc tat gag agt gaa atg tta gtt gcc tac tgt atg ttt cag ctg      257
Glu Cys Tyr Glu Ser Glu Met Leu Val Ala Tyr Cys Met Phe Gln Leu
      25      30      35
gag tgc tgt gtc aaa gga aat cct gca ccc tgacataaga aaccaatgaa      307
Glu Cys Cys Val Lys Gly Asn Pro Ala Pro
      40      45
tggccactat cctgtaggcc cttgattctg ccatctttca caaaaccagg gaatttagat      367
caaactgtga caccatgatg tgtccatgac tactggtttt tagcattttt ataggccagc      427
agactcttgt ggtctttaat ttaaagagct gagctgtagc cttctttaaa agagctcggt      487
ttttcacaaa aacaatgtag aagatatttt ctcacctcaa cgtgatgtcc agtgtgctca      547
tcagcacctg tttctccctc taatcataga ggatattctt attatttaga aaggcttcaa      607
gggaaacaac ttttggcacc taagtctgtg cctaccttcg cttcagcttc gcatttccca      667
tttctgtgaa attcccaact ttagagaagc agatttgcca tggccttctg acaaccttgt      727
acatctctca cataaaccgc ataggcaggg cttaactaca ggctggcccg agtctggact      787
gagtctgacc ctgaagttcc tttggaacag gagaggccat cttgtgatgg gctggaacaa      847
ggtaatttct catccacctc cctagtttca gttgagcaat ggaacttccc acctgagccc      907
ctagggttca gctacaggct ataagactgc cgtcctgtgg tttagtgttg gttccttagc      967
agcagagtga tgccacctct gctgcccgtc atctgactcc tctggatggg tggtatcctg      1027
tggcttaaga gctaacacca tgctgatctt gctttgctat atgtgtaact aataaactgc      1087
ctaatgcaa aaaaaaaaaa aaa      1110

<210> 33
<211> 623
<212> DNA
<213> Homo Sapiens

<220>
<221> CDS
<222> 49..447

<220>
<221> sig_peptide
<222> 49..111
<223> Von Heijne matrix
      score 5.00
      seq LIVIFFYCWLSSS/HE

<220>
<221> polyA_signal
<222> 579..584

<220>
<221> polyA_site
<222> 602..623

<400> 33
attagaatth tctttctcaa attaaagggt tgagaaattc gtgatgag atg tcc tgt      57
                                Met Ser Cys
                                -20
tcc cta aag ttt act ttg att gta att ttt ttt tac tgt tgg ctt tca      105
Ser Leu Lys Phe Thr Leu Ile Val Ile Phe Phe Tyr Cys Trp Leu Ser
      -15      -10      -5
tcc agc cat gag gag tta gaa ggt ggt aca tcg aag tct ttt gac etc      153

```



35

```

Ser Ser His Glu Glu Leu Glu Gly Gly Thr Ser Lys Ser Phe Asp Leu
  1      5      10
cat aca gtg att atg ctt gtc atc gct ggt ggt atc ctg gcg gcc ttg      201
His Thr Val Ile Met Leu Val Ile Ala Gly Gly Ile Leu Ala Ala Leu
15      20      25      30
ctc ctg ctg ata gtt gtc gtg ctc tgt ctt tac ttc aaa ata cac aac      249
Leu Leu Leu Ile Val Val Leu Cys Leu Tyr Phe Lys Ile His Asn
      35      40      45
gcg cta aaa gct gca aag gaa cct gaa gct gtg gct gta aaa aat cac      297
Ala Leu Lys Ala Ala Lys Glu Pro Glu Ala Val Ala Val Lys Asn His
      50      55      60
aac cca gac aag gtg tgg tgg gcc aag aac agc cag gcc aaa acc att      345
Asn Pro Asp Lys Val Trp Trp Ala Lys Asn Ser Gln Ala Lys Thr Ile
      65      70      75
gcc acg gag tct tgt cct gcc ctg cag tgc tgt gaa gga tat aga atg      393
Ala Thr Glu Ser Cys Pro Ala Leu Gln Cys Cys Glu Gly Tyr Arg Met
      80      85      90
tgt gcc agt ttt gat tcc ctg cca cct tgc tgt tgc gac ata aat gag      441
Cys Ala Ser Phe Asp Ser Leu Pro Pro Cys Cys Cys Asp Ile Asn Glu
95      100      105      110
ggc ctc tgagtttagga aaggtgggca caaaaatcct catgagcaat acttcttagt      497
Gly Leu
agattgtttt gttattcaaa tcaagttcta gtgtttttat gtgagattat ataatttaca      557
gtgttgtttt atatactttt gaataaatgt acactattaa aaataaaaaa aaaaaaaaaat      617
gccaaa      623

```

<210> 34  
 <211> 657  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 199..618

<220>  
 <221> sig\_peptide  
 <222> 199..408  
 <223> Von Heijne matrix  
       score 3.90  
       seq FKVLTPQLSLLWG/CD

<220>  
 <221> polyA\_signal  
 <222> 626..631

<220>  
 <221> polyA\_site  
 <222> 643..657

```

<400> 34
aactggatag agtactgcc ccttcagccc atggagaaag gcaaatgcct ccttcagagt      60
ctacctaata ctttctcaga taaataagca tgaagaaaag tcaaagtcca ttctagctct      120
aaaataagga atgaaatggt ttcctgatat gattttttgt tttcatctga taataatttt      180
atatatcaca gaaacagc atg gtt ctt act aaa cct ctt caa aga aat ggc      231
          Met Val Leu Thr Lys Pro Leu Gln Arg Asn Gly
          -70          -65          -60
agc atg atg agc ttt gaa aat gtg aaa gaa aag agc aga gaa gga ggg      279
Ser Met Met Ser Phe Glu Asn Val Lys Glu Lys Ser Arg Glu Gly Gly
          -55          -50          -45
ccc cat gca cac aca ccc gaa gaa gaa ttg tgt ttc gtg gta aca cac      327
Pro His Ala His Thr Pro Glu Glu Glu Leu Cys Phe Val Val Thr His

```

36

```

          -40          -35          -30
tac cct cag gtt cag acc aca ctc aac ctg ttt ttc cat ata ttc aag      375
Tyr Pro Gln Val Gln Thr Thr Leu Asn Leu Phe Phe His Ile Phe Lys
          -25          -20          -15
gtt ctt act caa cca ctt tcc ctt ctg tgg ggt tgt gat cag aag cct      423
Val Leu Thr Gln Pro Leu Ser Leu Leu Trp Gly Cys Asp Gln Lys Pro
          -10          -5          1          5
cgt act gtt cct acc ctt gga aac ggc gca tgg gat acc tgc caa caa      471
Arg Thr Val Pro Thr Leu Gly Asn Gly Ala Trp Asp Thr Cys Gln Gln
          10          15          20
cac ata cgc act tca tca tgg aca gca aac aca ctc gtc att caa aac      519
His Ile Arg Thr Ser Ser Trp Thr Ala Asn Thr Leu Val Ile Gln Asn
          25          30          35
cag cat tca cgg gaa agc act gtt tct gtt tgc ctt ttt atg tta atc      567
Gln His Ser Arg Glu Ser Thr Val Ser Val Cys Leu Phe Met Leu Ile
          40          45          50
cgc atg caa cat att ttg aaa aca gat aca ctt caa cag ttc aga ata      615
Arg Met Gln His Ile Leu Lys Thr Asp Thr Leu Gln Gln Phe Arg Ile
          55          60          65
tgc tagtactaat aaaaccaaca tggtaaaaaa aaaaaaaaaa      657
Cys
70

```

<210> 35  
 <211> 1137  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 271..969

<220>  
 <221> sig\_peptide  
 <222> 271..366  
 <223> Von Heijne matrix  
       score 5.60  
       seq WMGLACFRSLAAS/SP

<220>  
 <221> polyA\_signal  
 <222> 1092..1097

<220>  
 <221> polyA\_site  
 <222> 1123..1137

```

<400> 35
aaaaaccttt caagtgcccc ctcttttctt taaagtcttt tatagggggtc cccttcttgg      60
ccatctccat cctgtgagtc aggactgaaa gggcacagac aggtcactgc cagcattgtt      120
ggggcaagcc tgcaagcacg catcactggg gatctgacat gacaatggcc gcctgcccc      180
tctgagggtt acaggactta cccagtgagg aagcagctaa gcaggtctga ccagccgacc      240
tggaacctggc caagggtcct gtcattccctc atg gcc acc ccg cca ttc cgg ctg      294
                               Met Ala Thr Pro Pro Phe Arg Leu
                               -30          -25
ata agg aag atg ttt tcc ttc aag gtg agc aga tgg atg ggg ctt gcc      342
Ile Arg Lys Met Phe Ser Phe Lys Val Ser Arg Trp Met Gly Leu Ala
          -20          -15          -10
tgc ttc cgg tcc ctg gcg gca tcc tct ccc agt att cgc cag aag aaa      390
Cys Phe Arg Ser Leu Ala Ala Ser Ser Pro Ser Ile Arg Gln Lys Lys
          -5          1          5
cta atg cac aag ctg cag gag gaa aag gct ttt cgc gaa gag atg aaa      438

```

37

```

Leu Met His Lys Leu Gln Glu Glu Lys Ala Phe Arg Glu Glu Met Lys
  10          15          20
att ttt cgt gaa aaa ata gag gac ttc agg gaa gag atg tgg act ttc      486
Ile Phe Arg Glu Lys Ile Glu Asp Phe Arg Glu Glu Met Trp Thr Phe
 25          30          35          40
cga ggc aag atc cat gct ttc cgg ggc cag atc ctg ggt ttt tgg gaa      534
Arg Gly Lys Ile His Ala Phe Arg Gly Gln Ile Leu Gly Phe Trp Glu
          45          50          55
gag gag aga cct ttc tgg gaa gag gag aaa acc ttc tgg aaa gag gaa      582
Glu Glu Arg Pro Phe Trp Glu Glu Glu Lys Thr Phe Trp Lys Glu Glu
          60          65          70
aaa tcc ttc tgg gaa atg gaa aag tct ttc agg gag gaa gag aaa act      630
Lys Ser Phe Trp Glu Met Glu Lys Ser Phe Arg Glu Glu Glu Lys Thr
          75          80          85
ttc tgg aaa aag tac cgc act ttc tgg aag gag gat aag gcc ttc tgg      678
Phe Trp Lys Lys Tyr Arg Thr Phe Trp Lys Glu Asp Lys Ala Phe Trp
          90          95          100
aaa gag gac aat gcc tta tgg gaa aga gac cgg aac ctt ctt cag gag      726
Lys Glu Asp Asn Ala Leu Trp Glu Arg Asp Arg Asn Leu Leu Gln Glu
          105          110          115          120
gac aag gcc ctg tgg gag gaa gaa aag gcc ctg tgg gta gag gaa aga      774
Asp Lys Ala Leu Trp Glu Glu Glu Lys Ala Leu Trp Val Glu Glu Arg
          125          130          135
gcc ctc ctt gag ggg gag aaa gcc ctg tgg gaa gat aaa acg tcc ctc      822
Ala Leu Leu Glu Gly Glu Lys Ala Leu Trp Glu Asp Lys Thr Ser Leu
          140          145          150
tgg gag gaa gag aat gcc ctc tgg gag gaa gag agg gcc ttc tgg atg      870
Trp Glu Glu Glu Asn Ala Leu Trp Glu Glu Glu Arg Ala Phe Trp Met
          155          160          165
gag aac aat ggc cac att gcc gga gag cag atg ctc gaa gat ggg ccc      918
Glu Asn Asn Gly His Ile Ala Gly Glu Gln Met Leu Glu Asp Gly Pro
          170          175          180
cac aac gcc aac aga ggg cag cgc ttg ctg gcc ttc tcc cga ggc agg      966
His Asn Ala Asn Arg Gly Gln Arg Leu Leu Ala Phe Ser Arg Gly Arg
          185          190          195          200
gcg tagccagcat gcaggtgcag ggccctgtgg tccagactcc cctggggttg      1019
Ala
gattcaagtc caggggtgagc ccatgtgctg gagaaaatac acactcattg gtctccttgc      1079
tttgaaagat ccaataaagt cctgaggcaa gggttggaac accaaaaaaa aaaaaaaa      1137

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&lt;210&gt; 36

&lt;211&gt; 636

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 192..440

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 192..278

&lt;223&gt; Von Heijne matrix

score 5.20

seq VVFM TVAAGGASS/FA

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 590..595

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 622..636

&lt;400&gt; 36

```

aaaagcgagt caggtccctc gcgctcccg cccacgcgcg tgaccagagc gcgctggccc      60
ggcccacccg gggcggttgt ggctcgtata tataaggtgg ggaggccgcc ggcccgttcg      120
gttcggggcg ttaccatcgt ccgtgcgcac cgcccggcgt ccagatttgg caattcttcg      180
ctgaagtcat c atg agc ttt ttc caa ctc ctg atg aaa agg aag gaa ctc      230
                Met Ser Phe Phe Gln Leu Leu Met Lys Arg Lys Glu Leu
                -25                                -20

att ccc ttg gtg gtg ttc atg act gtg gcg gcg ggt gga gcc tca tct      278
Ile Pro Leu Val Val Phe Met Thr Val Ala Ala Gly Gly Ala Ser Ser
      -15                                -10                                -5

ttc gct gtg tat tct ctt tgg aaa acc gat gtg atc ctt gat cga aaa      326
Phe Ala Val Tyr Ser Leu Trp Lys Thr Asp Val Ile Leu Asp Arg Lys
1      5      10      15

aaa aat cca gaa cct tgg gaa act gtg gac cct act gta cct caa aag      374
Lys Asn Pro Glu Pro Trp Glu Thr Val Asp Pro Thr Val Pro Gln Lys
      20      25      30

ctt ata aca atc aac caa caa tgg aaa ccc att gaa gag ttg caa aat      422
Leu Ile Thr Ile Asn Gln Gln Trp Lys Pro Ile Glu Glu Leu Gln Asn
      35      40      45

gtc caa agg gtg acc aaa tgacgagccc tcgcctcttt cttctgaaga      470
Val Gln Arg Val Thr Lys
50

gtactctata aatctagtgg aaacatttct gcacaaacta gattctggac accagtgtgc      530
ggaaatgctt ctgctacatt tttagggttt gtctacattt tttgggctct ggataaggaa      590
ttaaaggagt gcagcaataa ctgcactgtc caaaaaaaaa aaaaaa      636

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&lt;210&gt; 37

&lt;211&gt; 818

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 59..703

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 59..181

&lt;223&gt; Von Heijne matrix

score 6.80

seq LVSCLSQSSALS/QS

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 783..788

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 804..818

&lt;400&gt; 37

```

gacatcttga gctgaagcag ggttttgagc cactgctgct gctgctgcca ttgtcacc      58
atg gtc tca gct ctg cgg gga gca ccc ctg atc agg gtg cac tca agc      106
Met Val Ser Ala Leu Arg Gly Ala Pro Leu Ile Arg Val His Ser Ser
      -40      -35      -30

cct gtt tct tct cct tct gtg agt gga cca cgg agg ctg gtg agc tgc      154
Pro Val Ser Ser Pro Ser Val Ser Gly Pro Arg Arg Leu Val Ser Cys
      -25      -20      -15      -10

ctg tca tcc caa agc tca gct ctg agc cag agt ggt ggt ggc tcc acc      202
Leu Ser Ser Gln Ser Ser Ala Leu Ser Gln Ser Gly Gly Gly Ser Thr

```

39

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      -5              1              5
tct gcc gcc ggc ata gaa gcc agg agc agg gct ctc aga agg cgg tgg      250
Ser Ala Ala Gly Ile Glu Ala Arg Ser Arg Ala Leu Arg Arg Arg Trp
      10              15              20
tgc cca gct ggg atc atg ttg ttg gcc ctg gtc tgt ctg ctc agc tgc      298
Cys Pro Ala Gly Ile Met Leu Ala Leu Val Cys Leu Leu Ser Cys
      25              30              35
ctg cta ccc tcc agt gag gcc aag ctc tac ggt cgt tgt gaa ctg gcc      346
Leu Leu Pro Ser Ser Glu Ala Lys Leu Tyr Gly Arg Cys Glu Leu Ala
      40              45              50              55
aga gtg cta cat gac ttc ggg ctg gac gga tac cgg gga tac agc ctg      394
Arg Val Leu His Asp Phe Gly Leu Asp Gly Tyr Arg Gly Tyr Ser Leu
      60              65              70
gct gac tgg gtc tgc ctt gct tat ttc aca agc ggt ttc aac gca gct      442
Ala Asp Trp Val Cys Leu Ala Tyr Phe Thr Ser Gly Phe Asn Ala Ala
      75              80              85
gct ttg gac tac gag gct gat ggg agc acc aac aac ggg atc ttc cag      490
Ala Leu Asp Tyr Glu Ala Asp Gly Ser Thr Asn Asn Gly Ile Phe Gln
      90              95              100
atc aac agc cgg agg tgg tgc agc aac ctc acc ccg aac gtc ccc aac      538
Ile Asn Ser Arg Arg Trp Cys Ser Asn Leu Thr Pro Asn Val Pro Asn
      105              110              115
gtg tgc cgg atg tac tgc tca gat ttg ttg aat cct aat ctc aag gat      586
Val Cys Arg Met Tyr Cys Ser Asp Leu Leu Asn Pro Asn Leu Lys Asp
      120              125              130              135
acc gtt atc tgt gcc atg aag ata acc caa gag cct cag ggt ctg ggt      634
Thr Val Ile Cys Ala Met Lys Ile Thr Gln Glu Pro Gln Gly Leu Gly
      140              145              150
tac tgg gag gcc tgg agg cat cac tgc cag gga aaa gac ctc act gaa      682
Tyr Trp Glu Ala Trp Arg His His Cys Gln Gly Lys Asp Leu Thr Glu
      155              160              165
tgg gtg gat ggc tgt gac ttc taggatggac ggaaccatgc acagcaggct      733
Trp Val Asp Gly Cys Asp Phe
      170
gggaaatgtg gtttggttcc tgacctaggc ttgggaagac aagccagcga ataaaggatg      793
gttgaacgtg aaaaaaaaaa aaaaaa      818

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&lt;210&gt; 38

&lt;211&gt; 1888

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 139..1389

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 139..198

&lt;223&gt; Von Heijne matrix

score 5.00

seq HLLAGFCVWVVLG/WV

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1854..1859

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1873..1888

&lt;400&gt; 38

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ccccccagc tggaaccaag aaggttggtg ccccttctct ctgggtgtcc ttgtctctg      60
ctatcagggc acagtctca ggatgtttcg gggagaatag gagccagaac ctgagccccct      120
aagccattcc cctcacca atg atg ggg tcc cca gtg agt cat ctg ctg gcc      171
                Met Met Gly Ser Pro Val Ser His Leu Leu Ala
                -20                -15                -10
ggc ttc tgt gtg tgg gtc gtc ttg ggc tgg gta ggg ggc tca gtc ccc      219
Gly Phe Cys Val Trp Val Val Leu Gly Trp Val Gly Gly Ser Val Pro
                -5                1                5
aac ctg ggc cct gct gag cag gag cag aac cat tac ctg gcc cag ctg      267
Asn Leu Gly Pro Ala Glu Gln Glu Gln Asn His Tyr Leu Ala Gln Leu
                10                15                20
ttt ggc ctg tac ggc gag aat ggg acg ctg act gca ggg ggc ttg gcg      315
Phe Gly Leu Tyr Gly Glu Asn Gly Thr Leu Thr Ala Gly Gly Leu Ala
                25                30                35
cgg ctt ctc cac agc ctg ggg cta ggc cga gtt cag ggg ctt cgc ctg      363
Arg Leu Leu His Ser Leu Gly Leu Gly Arg Val Gln Gly Leu Arg Leu
40                45                50                55
gga cag cat ggg cct ctg act gga cgg gct gca tcc cca gct gca gac      411
Gly Gln His Gly Pro Leu Thr Gly Arg Ala Ala Ser Pro Ala Ala Asp
                60                65                70
aat tcc aca cac agg cca cag aac cct gag ctg agt gtg gat gtc tgg      459
Asn Ser Thr His Arg Pro Gln Asn Pro Glu Leu Ser Val Asp Val Trp
                75                80                85
gca ggg atg cct ctg ggt ccc tca ggg tgg ggt gac ctg gaa gag tca      507
Ala Gly Met Pro Leu Gly Pro Ser Gly Trp Gly Asp Leu Glu Glu Ser
90                95                100
aag gcc cct cac cta ccc cgt ggg cca gcc ccc tgc ggc ctg gac ctc      555
Lys Ala Pro His Leu Pro Arg Gly Pro Ala Pro Ser Gly Leu Asp Leu
105                110                115
ctt cac agg ctt ctg ttg ctg gac cac tca ttg gct gac cac ctg aat      603
Leu His Arg Leu Leu Leu Asp His Ser Leu Ala Asp His Leu Asn
120                125                130                135
gag gat tgt ctg aac ggc tcc cag ctg ctg gtc aat ttt ggc ttg agc      651
Glu Asp Cys Leu Asn Gly Ser Gln Leu Leu Val Asn Phe Gly Leu Ser
140                145                150
ccc gct gct cct ctg acc cct cgt cag ttt gct ctg ctg tgc cca gcc      699
Pro Ala Ala Pro Leu Thr Pro Arg Gln Phe Ala Leu Leu Cys Pro Ala
155                160                165
ctg ctt tat cag atc gac agc cgc gtc tgc atc ggc gct ccg gcc cct      747
Leu Leu Tyr Gln Ile Asp Ser Arg Val Cys Ile Gly Ala Pro Ala Pro
170                175                180
gca ccc cca ggg gat cta cta tct gcc ctg ctt cag agt gcc ctg gca      795
Ala Pro Pro Gly Asp Leu Leu Ser Ala Leu Leu Gln Ser Ala Leu Ala
185                190                195
gtc ctg ttg ctc agc ctc cct tct ccc cta tcc ctg ctg ctg ctg cgg      843
Val Leu Leu Leu Ser Leu Pro Ser Pro Leu Ser Leu Leu Leu Leu Arg
200                205                210                215
ctc ctg gga cct cgt cta cta cgg ccc ttg ctg ggc ttc ctg ggg gcc      891
Leu Leu Gly Pro Arg Leu Leu Arg Pro Leu Leu Gly Phe Leu Gly Ala
220                225                230
ctg gcg gtg ggc act ctt tgt ggg gat gca ctg cta cat ctg cta ccg      939
Leu Ala Val Gly Thr Leu Cys Gly Asp Ala Leu Leu His Leu Leu Pro
235                240                245
cat gca caa gaa ggg cgg cac gca gga cct ggc gga cta cca gag aag      987
His Ala Gln Glu Gly Arg His Ala Gly Pro Gly Gly Leu Pro Glu Lys
250                255                260
gac ctg ggc ccg ggg ctg tca gtg ctc gga ggc ctc ttc ctg ctc ttt      1035
Asp Leu Gly Pro Gly Leu Ser Val Leu Gly Gly Leu Phe Leu Leu Phe
265                270                275
gtg ctg gag aac atg ctg ggg ctt ttg cgg cac cga ggg ctc agg cca      1083
Val Leu Glu Asn Met Leu Gly Leu Leu Arg His Arg Gly Leu Arg Pro
280                285                290                295

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41

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aga tgc tgc agg cga aaa cga agg aat ctc gaa aca cgc aac ttg gat      1131
Arg Cys Cys Arg Arg Lys Arg Arg Asn Leu Glu Thr Arg Asn Leu Asp
                               300                               305                               310
ccg gag aat ggc agt ggg atg gcc ctt cag ccc cta cag gca gct cca      1179
Pro Glu Asn Gly Ser Gly Met Ala Leu Gln Pro Leu Gln Ala Ala Pro
                               315                               320                               325
gag cca ggg gct cag ggc cag agg gag aag aac agc cag cac cca cca      1227
Glu Pro Gly Ala Gln Gly Gln Arg Glu Lys Asn Ser Gln His Pro Pro
                               330                               335                               340
gct ctg gcc cct cct ggg cac caa ggc cac agt cat ggg cac cag ggt      1275
Ala Leu Ala Pro Pro Gly His Gln Gly His Ser His Gly His Gln Gly
                               345                               350                               355
ggc act gat atc acg tgg atg gtc ctc ctg gga gat ggt cta cac aac      1323
Gly Thr Asp Ile Thr Trp Met Val Leu Leu Gly Asp Gly Leu His Asn
360                               365                               370                               375
ctc act gat ggg ctg gcc ata ggt gct gcc ttc tct gat ggc ttc tcc      1371
Leu Thr Asp Gly Leu Ala Ile Gly Ala Ala Phe Ser Asp Gly Phe Ser
                               380                               385                               390
gcg gcc tca gta cca cct tagcgggtctt ctgccatgag ctgccccacg      1419
Ala Ala Ser Val Pro Pro
                               395
aactgggtga ctttgccatg ctgctccagt cagggctgtc ctttcggcgg ctgctgctgc      1479
tgagcctcgt gtctggagcc ctgggattgg ggggtgcagt cctgggggtg gggctcagcc      1539
tgggcctcgt cccctcact ccctgggtgt ttgggggtcac tgctgggggtc ttcctctatg      1599
tgccccttgt ggacatgcta ccagccctgc ttcgtcctcc ggagccctg cctacgcccc      1659
atgtgctcct gcaggggctg gggctgctgc tggggggcgg cctcatgctt gccataaccc      1719
tgctggagga gcggctactg cccgtgacca ctgagggtctg atggggccag tggaaagggg      1779
tcgggttgcc cttccttccc cccaaccaca ggaatggagg cgggacacag ggccagtagg      1839
agcaatagga ttttaataaa cagaacccat ccaaaaaaaa aaaaaaaaaa      1888

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&lt;210&gt; 39

&lt;211&gt; 1894

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 21..1118

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 21..89

&lt;223&gt; Von Heijne matrix

score 10.80

seq ALALLSAFSATQA/RK

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1858..1863

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1879..1894

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 1695

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 39

```

agacgtgagc agagcagata atg gca agc atg gct gcc gtg ctc acc tgg gct
Met Ala Ser Met Ala Ala Val Leu Thr Trp Ala

```

53

-15

ctg	gct	ctt	ctt	tca	gcg	ttt	tcg	gcc	acc	cag	gca	cgg	aaa	ggc	ttc	101
Leu	Ala	Leu	Leu	Ser	Ala	Phe	Ser	Ala	Thr	Gln	Ala	Arg	Lys	Gly	Phe	
		-10					-5					1				
tgg	gac	tac	ttc	agc	cag	acc	agc	ggg	gac	aaa	ggc	agg	gtg	gag	cag	149
Trp	Asp	Tyr	Phe	Ser	Gln	Thr	Ser	Gly	Asp	Lys	Gly	Arg	Val	Glu	Gln	
5				10					15					20		
atc	cat	cag	cag	aag	atg	gct	cgc	gag	ccc	gcg	acc	ctg	aaa	gac	agc	197
Ile	His	Gln	Gln	Lys	Met	Ala	Arg	Glu	Pro	Ala	Thr	Leu	Lys	Asp	Ser	
				25				30						35		
ctt	gag	caa	gac	ctc	aac	aat	atg	aac	aag	ttc	ctg	gaa	aag	ctg	agg	245
Leu	Glu	Gln	Asp	Leu	Asn	Asn	Met	Asn	Lys	Phe	Leu	Glu	Lys	Leu	Arg	
		40					45					50				
cct	ctg	agt	ggg	agc	gag	gct	cct	cgg	ctc	cca	cag	gac	ccg	gtg	ggc	293
Pro	Leu	Ser	Gly	Ser	Glu	Ala	Pro	Arg	Leu	Pro	Gln	Asp	Pro	Val	Gly	
		55					60					65				
atg	cgg	cgg	cag	ctg	cag	gag	gag	ttg	gag	gag	gtg	aag	gct	cgc	ctc	341
Met	Arg	Arg	Gln	Leu	Gln	Glu	Glu	Leu	Glu	Glu	Val	Lys	Ala	Arg	Leu	
		70				75				80						
cag	ccc	tac	atg	gca	gag	gcg	cac	gag	ctg	gtg	ggc	tgg	aat	ttg	gag	389
Gln	Pro	Tyr	Met	Ala	Glu	Ala	His	Glu	Leu	Val	Gly	Trp	Asn	Leu	Glu	
85				90						95					100	
ggc	ttg	cgg	cag	caa	ctg	aag	ccc	tac	acg	atg	gat	ctg	atg	gag	cag	437
Gly	Leu	Arg	Gln	Gln	Leu	Lys	Pro	Tyr	Thr	Met	Asp	Leu	Met	Glu	Gln	
				105				110						115		
gtg	gcc	ctg	cgc	gtg	cag	gag	ctg	cag	gag	cag	ttg	cgc	gtg	gtg	ggg	485
Val	Ala	Leu	Arg	Val	Gln	Glu	Leu	Gln	Glu	Gln	Leu	Arg	Val	Val	Gly	
				120			125						130			
gaa	gac	acc	aag	gcc	cag	ttg	ctg	ggg	ggc	gtg	gac	gag	gct	tgg	gct	533
Glu	Asp	Thr	Lys	Ala	Gln	Leu	Leu	Gly	Gly	Val	Asp	Glu	Ala	Trp	Ala	
		135					140					145				
ttg	ctg	cag	gga	ctg	cag	agc	cgc	gtg	gtg	cac	cac	acc	ggc	cgc	ttc	581
Leu	Leu	Gln	Gly	Leu	Gln	Ser	Arg	Val	Val	His	His	Thr	Gly	Arg	Phe	
		150				155						160				
aaa	gag	ctc	ttc	cac	cca	tac	gcc	gag	agc	ctg	gtg	agc	ggc	atc	ggg	629
Lys	Glu	Leu	Phe	His	Pro	Tyr	Ala	Glu	Ser	Leu	Val	Ser	Gly	Ile	Gly	
165				170						175				180		
cgc	cac	gtg	cag	gag	ctg	cac	cgc	agt	gtg	gct	ccg	cac	gcc	ccc	gcc	677
Arg	His	Val	Gln	Glu	Leu	His	Arg	Ser	Val	Ala	Pro	His	Ala	Pro	Ala	
				185				190						195		
agc	ccc	gcg	cgc	ctc	agt	cgc	tgc	gtg	cag	gtg	ctc					



43

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Asp Ser Gly Lys Val Leu Ser Lys Leu Gln Ala Arg Leu Asp Asp Leu
 310          315          320
tgg gaa gac atc act cac agc ctt cat gac cag ggc cac agc cat ctg      1109
Trp Glu Asp Ile Thr His Ser Leu His Asp Gln Gly His Ser His Leu
 325          330          335          340
ggg gac ccc tgaggatcta cctgcccagg cccattccca gctccttgct      1158
Gly Asp Pro
tggggagcct tggctctgag cctctagcat gggttcagtc ttgaaagtgg cctggtgggt      1218
ggagggtgga aggtcctgtg caggacaggg aggccaccaa aggggctgct gtctcctgca      1278
tatccagcct cctgcgactc cccaatctgg atgcattaca ttcaccaggc ttgcaaacc      1338
cagcctccca gtgctcattt gggaatgctc atgagttact ccattcaagg gtgagggagt      1398
agggagggag aggcaccatg catgtgggtg attatctgca agcctgtttg ccgtgatgct      1458
ggaagcctgt gccactacat cctggagttt ggctctagtc acttctggct gcctggtggc      1518
cactgctaca gctggtccac agagaggagc acttgctccc ccagggctgc catggcagct      1578
atcaggggaa tagaaggag aaagagaata tcatggggag aacatgtgat ggtgtgtgaa      1638
tatccctgct ggctctgatg ctggtgggtg cgaaagggtg gggctgtgat aggaganggc      1698
agagcccatg tttcctgaca tagctctaca cctaaataag ggactgaacc ctcccaactg      1758
tgggagctcc ttaaaccctc tggggagcat actgtgtgct ctccccatct ccagccctc      1818
cctctgggtt cccaagttga agcctagact tctggctcaa atgaaataga tgtttatgat      1878
aaaaaaaaa aaaaaa      1894

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&lt;210&gt; 40

&lt;211&gt; 1913

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 143..592

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 143..277

&lt;223&gt; Von Heijne matrix

score 5.90

seq VLVDLAILGQAYA/FA

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1877..1882

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1899..1913

&lt;400&gt; 40

```

atttttttgt gcctaagatg cccagtgcgt tgctgggttt ttctgctgct ctgggctct      60
ggacatgagg ccagaccttg tgaccttgtt ggcagtgggc agtggttga tgtgaggtcc      120
cagagacggc aggttcatca ag atg gtg ctc atg tgg acc agt ggt gac gcc      172
                               Met Val Leu Met Trp Thr Ser Gly Asp Ala
                               -45                               -40
ttc aag acg gcc tac ttc ctg ctg aag ggt gcc cct ctg cag ttc tcc      220
Phe Lys Thr Ala Tyr Phe Leu Leu Lys Gly Ala Pro Leu Gln Phe Ser
-35          -30          -25          -20
gtg tgc ggc ctg ctg cag gtg ctg gtg gac ctg gcc atc ctg ggg cag      268
Val Cys Gly Leu Leu Gln Val Leu Val Asp Leu Ala Ile Leu Gly Gln
          -15          -10          -5
gcc tac gcc ttc gcc cca ccc cca gaa gcc ggc gcc cca cgc cgt gca      316
Ala Tyr Ala Phe Ala Pro Pro Pro Glu Ala Gly Ala Pro Arg Arg Ala
          1          5          10
ccc cac tgg cac caa ggc cct ctg aca gtg ggg agg acg agg atg tgg      364
Pro His Trp His Gln Gly Pro Leu Thr Val Gly Arg Thr Arg Met Trp

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44

15	20	25	
gac cgc cag ccg cgg gca ctg gtg ggc cct gac ctc ccc gcg ggg agg			412
Asp Arg Gln Pro Arg Ala Leu Val Gly Pro Asp Leu Pro Ala Gly Arg			
30	35	40	45
gtg ggt gcc gtg gcc cct gca ggt gtg gca gag atg ggg cac ggg cat			460
Val Gly Ala Val Ala Pro Ala Gly Val Ala Glu Met Gly His Gly His			
	50	55	60
tgg ggt ctc cat cag cct ctg tgg ggt gtc tca ggg tgg gca gtg ggg			508
Trp Gly Leu His Gln Pro Leu Trp Gly Val Ser Gly Trp Ala Val Gly			
	65	70	75
gtg ggg ctg gga cgc tgt ttg tgc tca gcg ggg aca gcc agg gtt gat			556
Val Gly Leu Gly Arg Cys Leu Cys Ser Ala Gly Thr Ala Arg Val Asp			
	80	85	90
ctg gcc ccg agg gtt ttg gat gtt ttt agg atg aca taaaaagcaa			602
Leu Ala Pro Arg Val Leu Asp Val Phe Arg Met Thr			
95	100	105	
gtgtttttccc catttctctct tatgaaacac cgtctgagcc caagggtacac attgggcgggc			662
ctgcaggaac ctgctccagg tggacacacg ggccagcagc cgcgaacctt gaagctgggg			722
tgaccgcagg agacctgtga aggcctgtga ggcgagccct cgaccccggtg acacctggc			782
cagacacctt gcttggactg ggggtggcctc tgctaccagc ggggtctggca cgggggaggg			842
ctggggcttt ctctgcctgg tacacacgga aaggcggtg tgcggacgca gggtcaccgt			902
gctccgggtt ttctgacagt cgggtgtttcc tgggcctttg gagtggctgc gaggcctgaa			962
cgcttctgtg atccgctgtg tccagcccggt ctgagcatcg ccagggctag ctcatgctgc			1022
tcttgtcagc ctctggttct cctcgagtcc ttggggacgt ggcagatgcc agcgaccatc			1082
agacaacgtg gaggccctca tgggcaatgg ctgagggggc cgggctgagg ctgtgcacat			1142
gcagtctgca cgccactctt gggctctgct ggccgagatc cccttccttc tgggtgcaga			1202
ctgcacctcc ggatgcagtt ttgatgtcca tcttccagga gagagacggt ctccgggtcca			1262
gggagtggag ggggctgccc ctgccgtgca ggtcctggcc gatggcgcc taccctgctg			1322
ccctgggctt ttggcctgaa gcaaattcct gagtgggggg tactggggcc tgccgcaccc			1382
tgctctgtcc actgcccacc cccgtgtgct ggctccctca cttctggctg cagtgggagc			1442
cgccagtctg acccttgtca ccgcacgctc tgccccacc ccgttgcaag aggtcacacc			1502
atgtcagcag ccttgcactg accgcagccg gccccaggc ctcagagttc tggatgcttc			1562
cgtgcggctc caacaggcat cgtcttcctt tccgcaggtg gaggggccgc ttccgcagg			1622
catctgagct ctgtgccggg gccgtggcca tgggaagatg ttccacgctg cctcctctc			1682
gagttttcct cggaacact cttgaatgtc tgagtgaggg tctgtcttag ctctttggcc			1742
tgtgagatgc tttgaaaatt tttatttttt taagatgaag caagatgtct gtagcggtaa			1802
ttgcctcaca ttaactgtc gccgactgca ggcgcagtga ctgctgaatg taccctgtgt			1862
ggcgacttgg aatcaataaa ccatttgtgg atcctaaaaa aaaaaaaaaa a			1913

<210> 41  
 <211> 1744  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 76..999

<220>  
 <221> sig\_peptide  
 <222> 76..279  
 <223> Von Heijne matrix  
 score 5.10  
 seq LSLPVCTVSLVSS/VS

<220>  
 <221> polyA\_signal  
 <222> 1711..1716

<220>  
 <221> polyA\_site  
 <222> 1729..1744

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 336

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 41

```

aagttgagggc caccctgggtg gcaccaaagc cctctcaggc aggcagaccc agggcctccc      60
cgccacacct tggttc atg gat ttt gtc gct gga gcc atc gga ggc gtc tgc      111
               Met Asp Phe Val Ala Gly Ala Ile Gly Gly Val Cys
               -65                               -60

ggt gtt gct gtg ggc tac ccc ctg gac acg gtg aag gtc agg atc cag      159
Gly Val Ala Val Gly Tyr Pro Leu Asp Thr Val Lys Val Arg Ile Gln
-55                               -50                               -45

acg gag cca aag tac aca ggc atc tgg cac tgc gtc cgg gat acg tat      207
Thr Glu Pro Lys Tyr Thr Gly Ile Trp His Cys Val Arg Asp Thr Tyr
-40                               -35                               -30                               -25

cac cga gag cgc gtg tgg ggc ttc tac cgg ggc ctc tcg ctg ccc gtg      255
His Arg Glu Arg Val Trp Gly Phe Tyr Arg Gly Leu Ser Leu Pro Val
               -20                               -15                               -10

tgc acg gtg tcc ctg gta tct tcc gtg tct ttt ggc acc tac cgc cac      303
Cys Thr Val Ser Leu Val Ser Ser Val Ser Phe Gly Thr Tyr Arg His
               -5                               1                               5

tgc ctg gcg cac atc tgc cgg ctc cgg tac ggn aac cct gac gcc aag      351
Cys Leu Ala His Ile Cys Arg Leu Arg Tyr Gly Asn Pro Asp Ala Lys
10                               15                               20

ccc acc aag gcc gac atc acg ctc tcg gga tgc gcc tcc ggc ctc gtc      399
Pro Thr Lys Ala Asp Ile Thr Leu Ser Gly Cys Ala Ser Gly Leu Val
25                               30                               35                               40

cgc gtg ttc ctg acg tcg ccc act gag gtg gcc aaa gtc cgc ttg cag      447
Arg Val Phe Leu Thr Ser Pro Thr Glu Val Ala Lys Val Arg Leu Gln
               45                               50                               55

acg cag aca cag gcg cag aag cag cag cgg ctg ctt tcg gcc tcg ggg      495
Thr Gln Thr Gln Ala Gln Lys Gln Gln Arg Leu Leu Ser Ala Ser Gly
               60                               65                               70

ccg ttg gct gtg ccc ccc atg tgt cct gtg ccc cca gcc tgc cca gag      543
Pro Leu Ala Val Pro Pro Met Cys Pro Val Pro Pro Ala Cys Pro Glu
75                               80                               85

ccc aag tac cgc ggg cca ctg cac tgc ctg gcc acg gta gcc cgt gag      591
Pro Lys Tyr Arg Gly Pro Leu His Cys Leu Ala Thr Val Ala Arg Glu
90                               95                               100

gag ggg ctg tgc ggc ctc tac aag ggc agc tcg gcc ctg gtc tta cgg      639
Glu Gly Leu Cys Gly Leu Tyr Lys Gly Ser Ser Ala Leu Val Leu Arg
105                               110                               115                               120

gac ggc cac tcc ttt gcc acc tac ttc ctt tcc tac gcg gtc ctc tgc      687
Asp Gly His Ser Phe Ala Thr Tyr Phe Leu Ser Tyr Ala Val Leu Cys
               125                               130                               135

gag tgg ctc agc ccc gct ggc cac agc cgg cca gat gtc ccg ggc gtg      735
Glu Trp Leu Ser Pro Ala Gly His Ser Arg Pro Asp Val Pro Gly Val
               140                               145                               150

ctg gtg gcc ggg ggc tgt gca gga gtc ctg gcc tgg gct gtg gcc acc      783
Leu Val Ala Gly Gly Cys Ala Gly Val Leu Ala Trp Ala Val Ala Thr
155                               160                               165

ccc atg gac gtg atc aag tcg aga ctg cag gca gac ggg cag ggc cag      831
Pro Met Asp Val Ile Lys Ser Arg Leu Gln Ala Asp Gly Gln Gly Gln
170                               175                               180

agg cgc tac cgg ggt ctc ctg cac tgt atg gtg acc agc gtt cga gag      879
Arg Arg Tyr Arg Gly Leu Leu His Cys Met Val Thr Ser Val Arg Glu
185                               190                               195                               200

gag gga ccc cgg gtc ctt ttc aag ggg ctg gta ctc aat tgc tgc cgc      927
Glu Gly Pro Arg Val Leu Phe Lys Gly Leu Val Leu Asn Cys Cys Arg
               205                               210                               215

```

46

```

gcc ttc cct gtc aac atg gtg gtc ttc gtc gcc tat gag gca gtg ctg      975
Ala Phe Pro Val Asn Met Val Val Phe Val Ala Tyr Glu Ala Val Leu
                220                225                230
agg ctc gcc cgg ggt ctg ctc aca tagccggtcc ccacgccag cgccccaccc      1029
Arg Leu Ala Arg Gly Leu Leu Thr
                235                240
accagcagct gctggagggtc gtagtgggtg gaggaggcaa ggggtagtgt ggctgggttc      1089
gggacccac agggccattg cccaggagaa tgaggagcct ccctgcagtg ttgtcgggcg      1149
aggcctaagc tcgccctgcc cagctactga cctcaggctg aggggcccgc cagccatcag      1209
ccagggttg cctagggtgg caggagccag ggaggagtgg gcctctttga tgagagcggt      1269
gagttgcatg gagtcgggtt ttcattccag cctcccatg gccctcgctt cccatgtctt      1329
tgaagcacc ctcaggaggag tcaggtgtgt gctcagccac cctctgcccc attcctagac      1389
cctcaccgcc accactgttc ctgtgtcttc atgagctgtc ccttacaggc aggggcttcc      1449
cacaggctgg gggcctcggg gcggggagca tgagctgggc tggcaccacg actgaggggt      1509
cccgccccgg cttcttcccc acagcagget gctcagaggg ggtgctgccc ggactgccat      1569
gcccacctga gaggggacct ggggtggcgt cctcgccggg ttagggaatt tggggtgagg      1629
ttcctcagga gccctcactc tgctgtgga cgctgcacct gccacttaaa gaccccaaag      1689
actctgttgg gaactgttgt caataaaatg tttctgagga aaaaaaaaaa aaaaa      1744

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<210> 42  
 <211> 946  
 <212> DNA  
 <213> Homo Sapiens

<220>  
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 <222> 123..464

<220>  
 <221> sig\_peptide  
 <222> 123..269  
 <223> Von Heijne matrix  
 score 4.90  
 seq PSLAAGLLFGSLA/GL

<220>  
 <221> polyA\_signal  
 <222> 908..913

<220>  
 <221> polyA\_site  
 <222> 931..946

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<400> 42
aaatcgcggtt tccggagaga cctggctgct gtgtcccgcg gcttgcgctc cgtagtggac      60
tccgcggggcc ttcggcagat gcaggcctgg ggtagtctcc tttctggact gagaagagaa      120
ga atg gag aag ccc ctc ttc cca tta gtg cct ttg cat tgg ttt ggc      167
  Met Glu Lys Pro Leu Phe Pro Leu Val Pro Leu His Trp Phe Gly
                -45                -40                -35
ttt ggc tac aca gca ctg gtt gtt tct ggt ggg atc gtt ggc tat gta      215
Phe Gly Tyr Thr Ala Leu Val Val Ser Gly Gly Ile Val Gly Tyr Val
                -30                -25                -20
aaa aca ggc agc gtg ccg tcc ctg gct gca ggg ctg ctc ttc ggc agt      263
Lys Thr Gly Ser Val Pro Ser Leu Ala Ala Gly Leu Leu Phe Gly Ser
                -15                -10                -5
cta gcc ggc ctg ggt gct tac cag ctg tat cag gat cca agg aac gtt      311
Leu Ala Gly Leu Gly Ala Tyr Gln Leu Tyr Gln Asp Pro Arg Asn Val
                1                5                10
tgg ggt ttc cta gcc gct aca tct gtt act ttt gtt ggt gtt atg gga      359
Trp Gly Phe Leu Ala Ala Thr Ser Val Thr Phe Val Gly Val Met Gly
                15                20                25                30
atg aga tcc tac tac tat gga aaa ttc atg cct gta ggt tta att gca      407

```

47

Met	Arg	Ser	Tyr	Tyr	Tyr	Gly	Lys	Phe	Met	Pro	Val	Gly	Leu	Ile	Ala	
			35						40					45		
ggt	gcc	agt	ttg	ctg	atg	gcc	gcc	aaa	ggt	gga	ggt	cgt	atg	ttg	atg	455
Gly	Ala	Ser	Leu	Leu	Met	Ala	Ala	Lys	Val	Gly	Val	Arg	Met	Leu	Met	
			50					55					60			
aca	tct	gat	tagcagaagt	catgttccag	cttggactca	tgaaggatta										504
Thr	Ser	Asp														
			65													
aaaatctgca	tcttccacta	ttttcaatgt	attaagagaa	ataagtgcag	cattttttgca											564
tctgacattt	tacctaataaa	aaaaaagaca	ccaaatttgg	cggaggggtg	gaaaatcagt											624
tggtaccatt	ataaccctac	agaggtggtg	agcatgtaac	atgagcttat	tgagaccatc											684
atagagatcg	attcttgtat	attgatttta	tctctttctg	tatctatagg	taaatctcaa											744
gggtaaaatg	ttaggtgttg	acattgagaa	ccctgaaacc	ccattccctg	ctcagaggaa											804
cagtgtgaaa	aaaaatctct	tgagagattt	agaatatctt	ttcttttgct	catcttagac											864
cacagactga	ctttgaaatt	atgttaagtg	aaatatcaat	gaaaataaag	tttactataa											924
ataattaaaa	aaaaaaaaaa	aa														946

<210> 43  
 <211> 1622  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 85..1230

<220>  
 <221> sig\_peptide  
 <222> 85..129  
 <223> Von Heijne matrix  
 score 10.10  
 seq LLLPLALCILVLC/CG

<220>  
 <221> polyA\_signal  
 <222> 1589..1594

<220>  
 <221> polyA\_site  
 <222> 1607..1622

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aaagtctgcc	ttaaagagcc	ttacaagcca	gccagtcctt	gcagctccac	aaactgaccc												60
atcctgggcc	ttgttctcca	caga	atg	ggt	ctg	ctc	ctt	ccc	ctg	gca	ctc						111
			Met	Gly	Leu	Leu	Leu	Pro	Leu	Ala	Leu						
			-15					-10									
tgc	atc	cta	gtc	ctg	tgc	tgc	gga	gca	atg	tct	cca	ccc	cag	ctg	gcc		159
Cys	Ile	Leu	Val	Leu	Cys	Cys	Gly	Ala	Met	Ser	Pro	Pro	Gln	Leu	Ala		
	-5				1				5					10			
ctc	aac	ccc	tcg	gct	ctg	ctc	tcc	cgg	ggc	tgc	aat	gac	tca	gat	gtg		207
Leu	Asn	Pro	Ser	Ala	Leu	Leu	Ser	Arg	Gly	Cys	Asn	Asp	Ser	Asp	Val		
			15				20				25						
ctg	gca	gtt	gca	ggc	ttt	gcc	ctg	cgg	gat	att	aac	aaa	gac	aga	aag		255
Leu	Ala	Val	Ala	Gly	Phe	Ala	Leu	Arg	Asp	Ile	Asn	Lys	Asp	Arg	Lys		
			30				35				40						
gat	ggc	tat	gtg	ctg	aga	ctc	aac	cga	gtg	aac	gac	gcc	cag	gaa	tac		303
Asp	Gly	Tyr	Val	Leu	Arg	Leu	Asn	Arg	Val	Asn	Asp	Ala	Gln	Glu	Tyr		
			45				50				55						
aga	cgg	ggt	ggc	ctg	gga	tct	ctg	ttc	tat	ctt	aca	ctg	gat	gtg	cta		351
Arg	Arg	Gly	Gly	Leu	Gly	Ser	Leu	Phe	Tyr	Leu	Thr	Leu	Asp	Val	Leu		
			60				65				70						
gag	act	gac	tgc	cat	gtg	ctc	aga	aag	aag	gca	tgg	caa	gac	tgt	gga		399

Glu Thr Asp Cys His Val Leu Arg Lys Lys Ala Trp Gln Asp Cys Gly	
75 80 85 90	
atg agg ata ttt ttt gaa tca gtt tat ggt caa tgc aaa gca ata ttt	447
Met Arg Ile Phe Phe Glu Ser Val Tyr Gly Gln Cys Lys Ala Ile Phe	
95 100 105	
tat atg aac aac cca agt aga gtt ctc tat tta gct gct tat aac tgt	495
Tyr Met Asn Asn Pro Ser Arg Val Leu Tyr Leu Ala Ala Tyr Asn Cys	
110 115 120	
act ctt cgc cca gtt tca aaa aaa aag att tac atg acg tgc cct gac	543
Thr Leu Arg Pro Val Ser Lys Lys Lys Ile Tyr Met Thr Cys Pro Asp	
125 130 135	
tgc cca agc tcc ata ccc act gac tct tcc aat cac caa gtg ctg gag	591
Cys Pro Ser Ser Ile Pro Thr Asp Ser Ser Asn His Gln Val Leu Glu	
140 145 150	
gct gcc acc gag tct ctt gcg aaa tac aac aat gag aac aca tcc aag	639
Ala Ala Thr Glu Ser Leu Ala Lys Tyr Asn Asn Glu Asn Thr Ser Lys	
155 160 165 170	
cag tat tct ctc ttc aaa gtc acc agg gct tct agc cag tgg gtg gtc	687
Gln Tyr Ser Leu Phe Lys Val Thr Arg Ala Ser Ser Gln Trp Val Val	
175 180 185	
ggc cct tct tac ttt gtg gaa tac tta att aaa gaa tca cca tgt act	735
Gly Pro Ser Tyr Phe Val Glu Tyr Leu Ile Lys Glu Ser Pro Cys Thr	
190 195 200	
aaa tcc cag gcc agc agc tgt tca ctt cag tcc tcc gac tct gtg cct	783
Lys Ser Gln Ala Ser Ser Cys Ser Leu Gln Ser Ser Asp Ser Val Pro	
205 210 215	
gtt ggt ctt tgc aaa ggt tct ctg act cga aca cac tgg gaa aag ttt	831
Val Gly Leu Cys Lys Gly Ser Leu Thr Arg Thr His Trp Glu Lys Phe	
220 225 230	
gtc tct gtg act tgt gac ttc ttt gaa tca cag gct cca gcc act gga	879
Val Ser Val Thr Cys Asp Phe Phe Glu Ser Gln Ala Pro Ala Thr Gly	
235 240 245 250	
agt gaa aac tct gct gtt aac cag aaa cct aca aac ctt ccc aag gtg	927
Ser Glu Asn Ser Ala Val Asn Gln Lys Pro Thr Asn Leu Pro Lys Val	
255 260 265	
gaa gaa tcc cag cag aaa aac acc ccc cca aca gac tcc ccc tcc aaa	975
Glu Glu Ser Gln Gln Lys Asn Thr Pro Pro Thr Asp Ser Pro Ser Lys	
270 275 280	
gct ggg cca aga gga tct gtc caa tat ctt cct gac ttg gat gat aaa	1023
Ala Gly Pro Arg Gly Ser Val Gln Tyr Leu Pro Asp Leu Asp Asp Lys	
285 290 295	
aat tcc cag gaa aag ggc cct cag gag gcc ttt cct gtg cat ctg gac	1071
Asn Ser Gln Glu Lys Gly Pro Gln Glu Ala Phe Pro Val His Leu Asp	
300 305 310	
cta acc acg aat ccc cag gga gaa acc ctg gat att tcc ttc ctc ttc	1119
Leu Thr Thr Asn Pro Gln Gly Glu Thr Leu Asp Ile Ser Phe Leu Phe	
315 320 325 330	
ctg gag cct atg gag gag aag ctg gtg gtc ctg cct ttc ccc aaa gaa	1167
Leu Glu Pro Met Glu Glu Lys Leu Val Val Leu Pro Phe Pro Lys Glu	
335 340 345	
aaa gca cgc act gct gag tgc cca ggg cca gcc cag aat gcc agc cct	1215
Lys Ala Arg Thr Ala Glu Cys Pro Gly Pro Ala Gln Asn Ala Ser Pro	
350 355 360	
ctt gtc ctt ccg cca tgagaatcac acagagtctt ctgtaggggt atgggtgcgcc	1270
Leu Val Leu Pro Pro	
365	
gcatgacatg ggaggcgatg gggacgatgg acagagacag agcgtgcaca cgtagagtgg	1330
ctagtgaagg acgccttttt gactcttctt ggtctcagca tggtgactgg gattggaaat	1390
aatgagactg agccctcggc ttgggctgca ctctaccctg tacactgcct tgtaccctga	1450
gctgcatcac ctctaaact gagcagtctc ataccatgga gagatgcctc tcttatgtct	1510
tcagccactc acttataaag atacttatct tttcagcagt atatatgtgc tgaaatctca	1570
gcatgaaagc attgcatgag taaagatact ttccctaaaa aaaaaaaaaa aa	1622

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<210> 44
<211> 715
<212> DNA
<213> Homo Sapiens
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<220>  
<221> CDS  
<222> 29..664
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<220>
<221> sig_peptide
<222> 29..619
<223> Von Heijne matrix
      score 4.80
      seq SFFGASFLMGSLG/GM
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<220>
<221> polyA_signal
<222> 657..662
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<220>
<221> polyA_site
<222> 699..715
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<220>
<221> misc_feature
<222> 295,357
<223> n=a, g, c or t
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<220>  
<221> unsure  
<222> -88  
<223> Xaa = Ala,Asp,Gly,Val
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<220>  
<221> unsure  
<222> -109  
<223> Xaa = Asp,Glu
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[illegible]

50

```

tgg ctc tgt ggc tgt gnc ttc acc ctc gct gat gtc ctc ctg gga gcc      388
Trp Leu Cys Gly Cys Xaa Phe Thr Leu Ala Asp Val Leu Leu Gly Ala
          -90                      -85                      -80
acc ctg cac cgc ctc aag ttc ctg gga ctg tcc aag aaa tac tgg gaa      436
Thr Leu His Arg Leu Lys Phe Leu Gly Leu Ser Lys Lys Tyr Trp Glu
          -75                      -70                      -65
gat ggc agc cgg ccc aac ctg cag tcc ttc ttt gag agg gtc cag aga      484
Asp Gly Ser Arg Pro Asn Leu Gln Ser Phe Phe Glu Arg Val Gln Arg
          -60                      -55                      -50
cgc ttt gcc ttc cgg aaa gtc ctg ggt gac atc cac acc acc ctg ctg      532
Arg Phe Ala Phe Arg Lys Val Leu Gly Asp Ile His Thr Thr Leu Leu
          -45                      -40                      -35                      -30
tcg gcc gtc atc ccc aat gct ttc cgg ctg gtc aag agg aaa ccc cca      580
Ser Ala Val Ile Pro Asn Ala Phe Arg Leu Val Lys Arg Lys Pro Pro
          -25                      -20                      -15
tcc ttc ttc ggg gcg tcc ttc ctc atg ggc tcc ctg ggt ggg atg ggc      628
Ser Phe Phe Gly Ala Ser Phe Leu Met Gly Ser Leu Gly Gly Met Gly
          -10                      -5                      1
tac ttt gcc tac tgg tac ctc aag aaa aaa tac atc tagggccagg      674
Tyr Phe Ala Tyr Trp Tyr Leu Lys Lys Lys Tyr Ile
          5                      10                      15
cctggggcctt ggtgtctgac tgccaaaaaa aaaaaaaaaa a      715

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<210> 45  
 <211> 1549  
 <212> DNA  
 <213> Homo Sapiens

<220>  
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 <222> 18..878

<220>  
 <221> sig\_peptide  
 <222> 18..95  
 <223> Von Heijne matrix  
       score 6.30  
       seq GVGLVTLGLAVG/SY

<220>  
 <221> polyA\_signal  
 <222> 1500..1505

<220>  
 <221> polyA\_site  
 <222> 1533..1549

<220>  
 <221> misc\_feature  
 <222> 944  
 <223> n=a, g, c or t

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<400> 45
ggaaaaggcg ctccgtc atg ggg atc cag acg agc ccc gtc ctg ctg gcc      50
          Met Gly Ile Gln Thr Ser Pro Val Leu Leu Ala
          -25                      -20
tcc ctg ggg gtg ggg ctg gtc act ctg ctc ggc ctg gct gtg ggc tcc      98
Ser Leu Gly Val Gly Leu Val Thr Leu Leu Gly Leu Ala Val Gly Ser
          -15                      -10                      -5                      1
tac ttg gtt cgg agg tcc cgc cgg cct cag gtc act ctc ctg gac ccc      146
Tyr Leu Val Arg Arg Ser Arg Arg Pro Gln Val Thr Leu Leu Asp Pro
          5                      10                      15

```



51

```

aat gaa aag tac ctg cta cga ctg cta gac aag acg ctc tct gca cgg      194
Asn Glu Lys Tyr Leu Leu Arg Leu Leu Asp Lys Thr Leu Ser Ala Arg
      20                      25                      30
tcc cca ggc aaa cat atc tac ctc tcc acc cga att gat ggc agc ctg      242
Ser Pro Gly Lys His Ile Tyr Leu Ser Thr Arg Ile Asp Gly Ser Leu
      35                      40                      45
gtc atc agg cca tac act cct gtc acc agt gat gag gat caa ggc tat      290
Val Ile Arg Pro Tyr Thr Pro Val Thr Ser Asp Glu Asp Gln Gly Tyr
      50                      55                      60                      65
gtg gat ctt gtc atc aag gtc tac ctg aag ggt gtg cac ccc aaa ttt      338
Val Asp Leu Val Ile Lys Val Tyr Leu Lys Gly Val His Pro Lys Phe
      70                      75                      80
cct gag gga ggg aag atg tct cag tac ctg gat agc ctg aag gtt ggg      386
Pro Glu Gly Gly Lys Met Ser Gln Tyr Leu Asp Ser Leu Lys Val Gly
      85                      90                      95
gat gtg gtg gag ttt cgg ggg cca agc ggg ttg ctc act tac act gga      434
Asp Val Val Glu Phe Arg Gly Pro Ser Gly Leu Leu Thr Tyr Thr Gly
      100                      105                      110
aaa ggg cat ttt aac att cag ccc aac aag aaa tct cca cca gaa ccc      482
Lys Gly His Phe Asn Ile Gln Pro Asn Lys Lys Ser Pro Pro Glu Pro
      115                      120                      125
cga gtg gcg aag aaa ctg gga atg att gcc ggc ggg aca gga atc acc      530
Arg Val Ala Lys Lys Leu Gly Met Ile Ala Gly Gly Thr Gly Ile Thr
      130                      135                      140                      145
cca atg cta cag ctg atc cgg gcc atc ctg aaa gtc cct gaa gat cca      578
Pro Met Leu Gln Leu Ile Arg Ala Ile Leu Lys Val Pro Glu Asp Pro
      150                      155                      160
acc cag tgc ttt ctg ctt ttt gcc aac cag aca gaa aag gat atc atc      626
Thr Gln Cys Phe Leu Leu Phe Ala Asn Gln Thr Glu Lys Asp Ile Ile
      165                      170                      175
ttg cgg gag gac tta gag gaa ctg cag gcc cgc tat ccc aat cgc ttt      674
Leu Arg Glu Asp Leu Glu Glu Gln Ala Arg Tyr Pro Asn Arg Phe
      180                      185                      190
aag ctc tgg ttc act ctg gat cat ccc cca aaa gat tgg gcc tac agc      722
Lys Leu Trp Phe Thr Leu Asp His Pro Pro Lys Asp Trp Ala Tyr Ser
      195                      200                      205
aag ggc ttt gtg act gcc gac atg atc cgg gaa cac ctg ccc gct cca      770
Lys Gly Phe Val Thr Ala Asp Met Ile Arg Glu His Leu Pro Ala Pro
      210                      215                      220                      225
ggg gat gat gtg ctg gta ctg ctt tgt ggg cca ccc cca atg gtg cag      818
Gly Asp Asp Val Leu Val Leu Leu Cys Gly Pro Pro Pro Met Val Gln
      230                      235                      240
ctg gcc tgc cat ccc aac ttg gac aaa ctg ggc tac tca caa aag atg      866
Leu Ala Cys His Pro Asn Leu Asp Lys Leu Gly Tyr Ser Gln Lys Met
      245                      250                      255
cga ttc acc tac tgagcaccct ccagcttccc tggtgctgtt cgctgcagtt      918
Arg Phe Thr Tyr
      260
gttccccatc agtactcaag cactanaagc cttagattcc tttcctcaga gtttcaggtt      978
ttttcagtta catctagagc tgaaatctgg atagtagctg caggaacaat attcctgtag      1038
ccatggaaga gggccaaggc tcagtcactc cttggatggc ctcctaaatc tccccgtggc      1098
aacaggtcca ggagaggccc atggagcagt ctcttccatg gagtaagaag gaagggagca      1158
tgtacgcttg gtccaagatt ggctagttcc ttgatagcat cttactctca cttcttttgt      1218
gtctgtgatg aaaggaacag tctgtgcaat gggttttact taaacttcac tgttcaacct      1278
atgagcaaat ctgtatgtgt gagtataagt tgagcatagc atacttccag aggtggtcct      1338
atggagatgg caagaaagga ggaaatgatt tcttcagatc tcaaaggagt ctgaaatc      1398
atatttctgt gtgtgtctct ctcagccctt gccaggcta gagggaaaca gctactgata      1458
atcgaaaact gctgtttgtg gcaggaaccc ctggctgtgc aaataaatgg ggctgaggcc      1518
cctgtgtgat attcaaaaaa aaaaaaaaaa a      1549

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&lt;210&gt; 46

&lt;211&gt; 1328

<212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 73..1008

<220>  
 <221> sig\_peptide  
 <222> 73..147  
 <223> Von Heijne matrix  
       score 14.10  
       seq LLLLLLLTLLAFA/GY

<220>  
 <221> polyA\_signal  
 <222> 1286..1291

<220>  
 <221> polyA\_site  
 <222> 1312..1328

<400> 46  
 actgcgcgga tcggcgtccg cagcgggagg ctgctgagct gccttgaggt gcagtgttgg 60  
 ggatccagag cc atg tcg gac ctg cta cta ctg ggc ctg att ggg ggc ctg 111  
                   Met Ser Asp Leu Leu Leu Leu Gly Leu Ile Gly Gly Leu  
                   -25                  -20                  -15  
 act ctc tta ctg ctg ctg acg ctg cta gcc ttt gcc ggg tac tca ggg 159  
 Thr Leu Leu Leu Leu Leu Thr Leu Leu Ala Phe Ala Gly Tyr Ser Gly  
                   -10                  -5                  1  
 cta ctg gct ggg gtg gaa gtg agt gct ggg tca ccc ccc atc cgc aac 207  
 Leu Leu Ala Gly Val Glu Val Ser Ala Gly Ser Pro Pro Ile Arg Asn  
                   5                  10                  15                  20  
 gtc act gtg gcc tac aag ttc cac atg ggg ctc tat ggt gag act ggg 255  
 Val Thr Val Ala Tyr Lys Phe His Met Gly Leu Tyr Gly Glu Thr Gly  
                   25                  30                  35  
 cgg ctt ttc act gag agc tgc atc tct ccc aag ctc cgc tcc atc gct 303  
 Arg Leu Phe Thr Glu Ser Cys Ile Ser Pro Lys Leu Arg Ser Ile Ala  
                   40                  45                  50  
 gtc tac tat gac aac ccc cac atg gtg ccc cct gat aag tgc cga tgt 351  
 Val Tyr Tyr Asp Asn Pro His Met Val Pro Pro Asp Lys Cys Arg Cys  
                   55                  60                  65  
 gcc gtg ggc agc atc ctg agt gaa ggt gag gaa tcg ccc tcc cct gag 399  
 Ala Val Gly Ser Ile Leu Ser Glu Gly Glu Glu Ser Pro Ser Pro Glu  
                   70                  75                  80  
 ctc atc gac ctc tac cag aaa ttt ggc ttc aag gtg ttc tcc ttc ccg 447  
 Leu Ile Asp Leu Tyr Gln Lys Phe Gly Phe Lys Val Phe Ser Phe Pro  
                   85                  90                  95                  100  
 gca ccc agc cat gtg gtg aca gcc acc ttc ccc tac acc acc att ctg 495  
 Ala Pro Ser His Val Val Thr Ala Thr Phe Pro Tyr Thr Thr Ile Leu  
                   105                  110                  115  
 tcc atc tgg ctg gct acc cgc cgt gtc cat cct gcc ttg gag acc tac 543  
 Ser Ile Trp Leu Ala Thr Arg Arg Val His Pro Ala Leu Asp Thr Tyr  
                   120                  125                  130  
 atc aag gag cgg aag ctg tgt gcc tat cct cgg ctg gag atc tac cag 591  
 Ile Lys Glu Arg Lys Leu Cys Ala Tyr Pro Arg Leu Glu Ile Tyr Gln  
                   135                  140                  145  
 gaa gac cag atc cat ttc atg tgc cca ctg gca cgg cag gga gac ttc 639  
 Glu Asp Gln Ile His Phe Met Cys Pro Leu Ala Arg Gln Gly Asp Phe  
                   150                  155                  160  
 tat gtg cct gag atg aag gag aca gag tgg aaa tgg cgg ggg ctt gtg 687  
 Tyr Val Pro Glu Met Lys Glu Thr Glu Trp Lys Trp Arg Gly Leu Val

53

165	170	175	180	
gag gcc att gac acc cag gtg gat ggc aca gga gct gac aca atg agt				735
Glu Ala Ile Asp Thr Gln Val Asp Gly Thr Gly Ala Asp Thr Met Ser				
	185	190	195	
gac acg agt tct gta agc ttg gaa gtg agc cct ggc agc cgg gag act				783
Asp Thr Ser Ser Val Ser Leu Glu Val Ser Pro Gly Ser Arg Glu Thr				
	200	205	210	
tca gct gcc aca ctg tca cct ggg gcg agc agc cgt ggc tgg gat gac				831
Ser Ala Ala Thr Leu Ser Pro Gly Ala Ser Ser Arg Gly Trp Asp Asp				
	215	220	225	
ggg gac acc cgc agc gag cac agc tac agc gag tca ggt gcc agc ggc				879
Gly Asp Thr Arg Ser Glu His Ser Tyr Ser Glu Ser Gly Ala Ser Gly				
	230	235	240	
tcc tct ttt gag gag ctg gac ttg gag ggc gag ggg ccc tta ggg gag				927
Ser Ser Phe Glu Glu Leu Asp Leu Glu Gly Glu Gly Pro Leu Gly Glu				
	245	250	255	260
tca cgg ctg gac cct ggg act gag ccc ctg ggg act acc aag tgg ctc				975
Ser Arg Leu Asp Pro Gly Thr Glu Pro Leu Gly Thr Thr Lys Trp Leu				
	265	270	275	
tgg gag ccc act gcc cct gag aag ggc aag gag taacccatgg cctgcaccct				1028
Trp Glu Pro Thr Ala Pro Glu Lys Gly Lys Glu				
	280	285		
cctgcagtgc agttgctgag gaactgagca gactctccag cagactctcc agccctcttc				1088
ctccttcctc tgggggagga ggggttcctg agggacctga cttcccctgc tccaggcctc				1148
ttgctaagcc ttctcctcac tgccctttag gctcccaggg ccagaggagc cagggactat				1208
tttctgcacc agccccaggg gctgccaccc ctgttgtgtc tttttttcag actcacagtg				1268
gagcttcag gacccagaat aaagccaatg atttacttgt ttcaaaaaaa aaaaaaaaaa				1328

&lt;210&gt; 47

&lt;211&gt; 1515

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 165..842

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 165..251

&lt;223&gt; Von Heijne matrix

score 7.00

seq LASFAALVLVCRQ/RV

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1474..1479

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1500..1515

&lt;400&gt; 47

agtcgcggga tgcgcccggg agccacagcc tgaggccctc aggtctctgc aggtgtcgtg	60
gaggaacctc gcacctgcca tcctcttccc caatttgcca cttccagcag ctttagccca	120
tgaggaggat gtgaccggga ctgagtcagg agccctctgg aagc atg gag act gtg	176
	Met Glu Thr Val
gtg att gtt gcc ata ggt gtg ctg gcc acc atc ttt ctg gct tcg ttt	224
Val Ile Val Ala Ile Gly Val Leu Ala Thr Ile Phe Leu Ala Ser Phe	
-25 -20 -15 -10	
gca gcc ttg gtg ctg gtt tgc agg cag cgc tac tgc cgg ccg cga gac	272
Ala Ala Leu Val Leu Val Cys Arg Gln Arg Tyr Cys Arg Pro Arg Asp	

54

	-5	1	5	
ctg ctg cag cgc tat gat tct aag ccc att gtg gac ctc att ggt gcc				320
Leu Leu Gln Arg Tyr Asp Ser Lys Pro Ile Val Asp Leu Ile Gly Ala				
10 15 20				
atg gag acc cag tct gag ccc tct gag tta gaa ctg gac gat gtc gtt				368
Met Glu Thr Gln Ser Glu Pro Ser Glu Leu Glu Leu Asp Asp Val Val				
25 30 35				
atc acc aac ccc cac att gag gcc att ctg gag aat gaa gac tgg atc				416
Ile Thr Asn Pro His Ile Glu Ala Ile Leu Glu Asn Glu Asp Trp Ile				
40 45 50 55				
gaa gat gcc tcg ggt ctc atg tcc cac tgc att gcc atc ttg aag att				464
Glu Asp Ala Ser Gly Leu Met Ser His Cys Ile Ala Ile Leu Lys Ile				
60 65 70				
tgt cac act ctg aca gag aag ctt gtt gcc atg aca atg ggc tct ggg				512
Cys His Thr Leu Thr Glu Lys Leu Val Ala Met Thr Met Gly Ser Gly				
75 80 85				
gcc aag atg aag act tca gcc agt gtc agc gac atc att gtg gtg gcc				560
Ala Lys Met Lys Thr Ser Ala Ser Val Ser Asp Ile Ile Val Val Ala				
90 95 100				
aag cgg atc agc ccc agg gtg gat gat gtt gtg aag tcg atg tac cct				608
Lys Arg Ile Ser Pro Arg Val Asp Asp Val Val Lys Ser Met Tyr Pro				
105 110 115				
ccg ttg gac ccc aaa ctc ctg gac gca cgg acg act gcc ctg ctc ctg				656
Pro Leu Asp Pro Lys Leu Leu Asp Ala Arg Thr Thr Ala Leu Leu Leu				
120 125 130 135				
tct gtc agt cac ctg gtg ctg gtg aca agg aat gcc tgc cat ctg acg				704
Ser Val Ser His Leu Val Leu Val Thr Arg Asn Ala Cys His Leu Thr				
140 145 150				
gga ggc ctg gac tgg att gac cag tct ctg tcg gct gct gag gag cat				752
Gly Gly Leu Asp Trp Ile Asp Gln Ser Leu Ser Ala Ala Glu Glu His				
155 160 165				
ttg gaa gtc ctt cga gaa gca gcc cta gct tct gag cca gat aaa ggc				800
Leu Glu Val Leu Arg Glu Ala Ala Leu Ala Ser Glu Pro Asp Lys Gly				
170 175 180				
ctc cca ggc cct gaa ggc ttc ctg cag gag cag tct gca att				842
Leu Pro Gly Pro Glu Gly Phe Leu Gln Glu Gln Ser Ala Ile				
185 190 195				
tagtgccctac aggccagcag ctatagcatga agggccctgc cgccatccct ggatggctca				902
gcttagcctt ctactttttc ctatagagtt agttgttctc cacggctgga gatttcagct				962
gtgtgtgcat agtaaagcag gagatccccg tcagtttatg cctcttttgc agttgcaaac				1022
tgtggctggt gagtggcagt ctaatactac agttagggga gatgccattc actctctgca				1082
agaggagtat tgaaaactgg tggactgtca gctttattta gctcacctag tgttttcaag				1142
aaaattgagc caccgtctaa gaaatcaaga ggtttcacat taaaattaga atttctggcc				1202
tctctcgatc ggtcagaatg tgtggcaatt ctgatctgca ttttcagaag aggacaatca				1262
attgaaacta agtaggggtt tcttcttttg gcaagacttg tactctctca cctggcctgt				1322
ttcattttatt tgtattatct gcctgggtccc tgaggcgtct ggggtctctcc tctcccttgc				1382
aggtttgggt ttgaagctga ggaactacaa agttgatgat ttctttttta tctttatgcc				1442
tgcaatttta cctagctacc actaggtgga tagtaaattt atacttatgt ttccccaaa				1502
aaaaaaaaa aaa				1515

&lt;210&gt; 48

&lt;211&gt; 1622

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 31..1248

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 31..135

55

<223> Von Heijne matrix  
score 6.30  
seq TLLLFAAPFGLLG/EK

<220>  
<221> polyA\_signal  
<222> 1580..1585

<220>  
<221> polyA\_site  
<222> 1607..1622

<400> 48  
aacctcttcc gtcggctgaa ttgcggccgt atg cgc ggc tct gtg gag tgc acc 54  
Met Arg Gly Ser Val Glu Cys Thr  
-35 -30  
tgg ggt tgg ggg cac tgt gcc ccc agc ccc ctg ctc ctt tgg act cta 102  
Trp Gly Trp Gly His Cys Ala Pro Ser Pro Leu Leu Leu Trp Thr Leu  
-25 -20 -15  
ctt ctg ttt gca gcc cca ttt ggc ctg ctg ggg gag aag acc cgc cag 150  
Leu Leu Phe Ala Ala Pro Phe Gly Leu Leu Gly Glu Lys Thr Arg Gln  
-10 -5 1 5  
gtg tct ctg gag gtc atc cct aac tgg ctg ggc ccc ctg cag aac ctg 198  
Val Ser Leu Glu Val Ile Pro Asn Trp Leu Gly Pro Leu Gln Asn Leu  
10 15 20  
ctt cat ata cgg gca gtg ggc acc aat tcc aca ctg cac tat gtg tgg 246  
Leu His Ile Arg Ala Val Gly Thr Asn Ser Thr Leu His Tyr Val Trp  
25 30 35  
agc agc ctg ggg cct ctg gca gtg gta atg gtg gcc acc aac acc ccc 294  
Ser Ser Leu Gly Pro Leu Ala Val Val Met Val Ala Thr Asn Thr Pro  
40 45 50  
cac agc acc ctg agc gtc aac tgg agc ctc ctg cta tcc cct gag ccc 342  
His Ser Thr Leu Ser Val Asn Trp Ser Leu Leu Leu Ser Pro Glu Pro  
55 60 65  
gat ggg ggc ctg atg gtg ctc cct aag gac agc att cag ttt tct tct 390  
Asp Gly Gly Leu Met Val Leu Pro Lys Asp Ser Ile Gln Phe Ser Ser  
70 75 80 85  
gcc ctt gtt ttt acc agg ctg ctt gag ttt gac agc acc aac gtg tcc 438  
Ala Leu Val Phe Thr Arg Leu Leu Glu Phe Asp Ser Thr Asn Val Ser  
90 95 100  
gat acg gca gca aag cct ttg gga aga cca tat cct cca tac tcc ttg 486  
Asp Thr Ala Ala Lys Pro Leu Gly Arg Pro Tyr Pro Pro Tyr Ser Leu  
105 110 115  
gcc gat ttc tct tgg aac aac atc act gat tca ttg gat cct gcc acc 534  
Ala Asp Phe Ser Trp Asn Asn Ile Thr Asp Ser Leu Asp Pro Ala Thr  
120 125 130  
ctg agt gcc aca ttt caa ggc cac ccc atg aac gac cct acc agg act 582  
Leu Ser Ala Thr Phe Gln Gly His Pro Met Asn Asp Pro Thr Arg Thr  
135 140 145  
ttt gcc aat ggc agc ctg gcc ttc agg gtc cag gcc ttt tcc agg tcc 630  
Phe Ala Asn Gly Ser Leu Ala Phe Arg Val Gln Ala Phe Ser Arg Ser  
150 155 160 165  
agc cga cca gcc caa ccc cct cgc ctc ctg cac aca gca gac acc tgt 678  
Ser Arg Pro Ala Gln Pro Pro Arg Leu Leu His Thr Ala Asp Thr Cys  
170 175 180  
cag cta gag gtg gcc ctg att gga gcc tct ccc cgg gga aac cgt tcc 726  
Gln Leu Glu Val Ala Leu Ile Gly Ala Ser Pro Arg Gly Asn Arg Ser  
185 190 195  
ctg ttt ggg ctg gag gta gcc aca ttg ggc cag ggc cct gac tgc ccc 774  
Leu Phe Gly Leu Glu Val Ala Thr Leu Gly Gln Gly Pro Asp Cys Pro  
200 205 210  
tca atg cag gag cag cac tcc atc gac gat gaa tat gca ccg gcc gtc 822

56

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Ser Met Gln Glu Gln His Ser Ile Asp Asp Glu Tyr Ala Pro Ala Val
 215          220          225
ttc cag ttg gac cag cta ctg tgg ggc tcc ctc cca tca ggc ttt gca      870
Phe Gln Leu Asp Gln Leu Leu Trp Gly Ser Leu Pro Ser Gly Phe Ala
230          235          240          245
cag tgg cga cca gtg gct tac tcc cag aag ccg ggg ggc cga gaa tca      918
Gln Trp Arg Pro Val Ala Tyr Ser Gln Lys Pro Gly Gly Arg Glu Ser
          250          255          260
gcc ctg ccc tgc caa gct tcc cct ctt cat cct gcc tta gca tac tct      966
Ala Leu Pro Cys Gln Ala Ser Pro Leu His Pro Ala Leu Ala Tyr Ser
          265          270          275
ctt ccc cag tca ccc att gtc cga gcc ttc ttt ggg tcc cag aat aac      1014
Leu Pro Gln Ser Pro Ile Val Arg Ala Phe Phe Gly Ser Gln Asn Asn
          280          285          290
ttc tgt gcc ttc aat ctg acg ttc ggg gct tcc aca ggc cct ggc tat      1062
Phe Cys Ala Phe Asn Leu Thr Phe Gly Ala Ser Thr Gly Pro Gly Tyr
          295          300          305
tgg gac caa cac tac ctc agc tgg tgg atg ctc ctg ggt gtg ggc ttc      1110
Trp Asp Gln His Tyr Leu Ser Trp Ser Met Leu Leu Gly Val Gly Phe
          310          315          320          325
cct cca gtg gac ggc ttg tcc cca cta gtc ctg ggc atc atg gca gtg      1158
Pro Pro Val Asp Gly Leu Ser Pro Leu Val Leu Gly Ile Met Ala Val
          330          335          340
gcc ctg ggt gcc cca ggg ctc atg ctg cta ggg ggc ggc ttg gtt ctg      1206
Ala Leu Gly Ala Pro Gly Leu Met Leu Leu Gly Gly Gly Leu Val Leu
          345          350          355
ctg ctg cac cac aag aag tac tca gag tac cag tcc ata aat      1248
Leu Leu His His Lys Lys Tyr Ser Glu Tyr Gln Ser Ile Asn
          360          365          370
taaggcccg ctcctggagg gaaggacatt actgaacctg tcttgctgtg cctcgaaact      1308
ctggaggttg gagcatcaag ttccagcccc cttcactccc ccactctgct tttctgtgga      1368
acctcagagg ccagcctcga cttcctggag acccccaggt ggggcttcct tcatactttg      1428
ttgggggact ttggaggcgg gcaggggaca gggctattga taagggtccc ttggtgttgc      1488
cttcttgcac ctccacacat ttcccttgga tgggacttgc aggcctaaat gagaggcatt      1548
ctgactggtt ggctgccctg gaaggcaaga aaatagattt attttttttt cacagggcaa      1608
aaaaaaaaaa aaaa      1622

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&lt;210&gt; 49

&lt;211&gt; 1448

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 131..490

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 131..301

&lt;223&gt; Von Heijne matrix

score 5.30

seq AIALATVFLIGA/FL

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1411..1416

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1434..1448

&lt;400&gt; 49

57

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ctgatcccgct ctggggccgg ctgagtggca cttaagcggg ccatgccatg caaccttggg      60
cgctgccaac cgtgggcgag ctctgggtgt gcgggcggcc tcgcgcggcg ctccgctgtg      120
tcagcgtgtt atg atg ccg tcc cgt acc aac ctg gct act gga atc ccc      169
      Met Met Pro Ser Arg Thr Asn Leu Ala Thr Gly Ile Pro
      -55 -50 -45
agt agt aaa gtg aaa tat tca agg ctc tcc agc aca gac gat ggc tac      217
Ser Ser Lys Val Lys Tyr Ser Arg Leu Ser Ser Thr Asp Asp Gly Tyr
      -40 -35 -30
att gac ctt cag ttt aag aaa acc cct cct aag atc cct tat aag gcc      265
Ile Asp Leu Gln Phe Lys Lys Thr Pro Pro Lys Ile Pro Tyr Lys Ala
      -25 -20 -15
atc gca ctt gcc act gtg ctg ttt ttg att ggc gcc ttt ctc att att      313
Ile Ala Leu Ala Thr Val Leu Phe Leu Ile Gly Ala Phe Leu Ile Ile
      -10 -5 1
ata ggc tcc ctc ctg ctg tca ggc tac atc agc aaa ggg ggg gca gac      361
Ile Gly Ser Leu Leu Ser Gly Tyr Ile Ser Lys Gly Gly Ala Asp
5 10 15 20
cgg gcc gtt cca gtg ctg atc att ggc att ctg gtg ttc cta ccc gga      409
Arg Ala Val Pro Val Leu Ile Ile Gly Ile Leu Val Phe Leu Pro Gly
      25 30 35
ttt tac cac ctg cgc atc gct tac tat gca tcc aaa ggc tac cgt ggt      457
Phe Tyr His Leu Arg Ile Ala Tyr Tyr Ala Ser Lys Gly Tyr Arg Gly
      40 45 50
tac tcc tat gat gac att cca gac ttt gat gac tagcaccac cccatagctg      510
Tyr Ser Tyr Asp Asp Ile Pro Asp Phe Asp Asp
      55 60
aggaggagtc acagtggaac tgtcccagct ttaagatatc tagcagaaac tatagctgag      570
gactaaggaa ttctgcagct tgcagatgtt taagaaaata atggccagat tttttgggtc      630
cttcccaaag atgttaagtg aacctacagt tagctaatta ggacaagctc tatttttcat      690
ccctggggcc tgacaagttt ttccacagga atatgtatca tggagaata gaggttattc      750
tgtaattgaa aagtgttgcc tgccaccacc ctctgtagag ctgagcattt cttttaaata      810
gtcttcattg ccaatttggt cttgtagcaa atggaacaat gtggtatggc taatttctta      870
ttattaagta atttatttta aaaatatctg agtatattat cctgtacact tatccctacc      930
ttcatgttcc agtgaagac cttagtataa tcaaagatca gtgagttcat ctgtaatatt      990
ttttttactt gctttcttac tgacagcaac caggaatttt tttatcctgc agagcaagtt      1050
ttcaaaatgt aaatacttcc tctgtttaac agtccttgga ccattctgat ccagttcacc      1110
agtaggttgg acagcatata atttgcatca ttttgtccct tgtaaatcaa gatgttctgc      1170
agattattcc tttaacggcc ggacttttgg ctgtttccta atgaaacatg tagtggttat      1230
ttatagagt ttatagccgt attgctagca ccttgtagta tgatcatcatt ctgctcatga      1290
ttccaaggat cagcctggat gcctagagga ctagatcacc ttagtttgat tctatttttt      1350
agcttgcaaa aagtgactta tattccaaag aaattaaaat gttgaaatcc aaatcctaga      1410
aataaaatga gttaacttca aacaaaaaaa aaaaaaaa      1448

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&lt;210&gt; 50

&lt;211&gt; 894

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 61..690

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 61..168

&lt;223&gt; Von Heijne matrix

score 4.60

seq GTVVVLVAGTLCFA/WW

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 858..863

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 879..894

&lt;400&gt; 50

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acaccttcac ctgcgcccag ctccctgcmc gcctggacag cgctgctgc cgcctcccg      60
atg gcc ctg ccc cag atg tgt gac ggg agc cac ttg gcc tcc acc ctc      108
Met Ala Leu Pro Gln Met Cys Asp Gly Ser His Leu Ala Ser Thr Leu
-35 -30 -25
cgc tat tgc atg aca gtc agc ggc aca gtg gtt ctg gtg gcc ggg acg      156
Arg Tyr Cys Met Thr Val Ser Gly Thr Val Val Leu Val Ala Gly Thr
-20 -15 -10 -5
ctc tgc ttc gct tgg tgg agc gaa ggg gat gca acc gcc cag cct ggc      204
Leu Cys Phe Ala Trp Trp Ser Glu Gly Asp Ala Thr Ala Gln Pro Gly
1 5 10
cag ctg gcc cca ccc acg gag tat ccg gtg cct gag ggc ccc agc ccc      252
Gln Leu Ala Pro Pro Thr Glu Tyr Pro Val Pro Glu Gly Pro Ser Pro
15 20 25
ctg ctc agg tcc gtc agc ttc gtc tgc tgc ggt gca ggt ggc ctg ctg      300
Leu Leu Arg Ser Val Ser Phe Val Cys Cys Gly Ala Gly Gly Leu Leu
30 35 40
ctg ctc att ggc ctg ctg tgg tcc gtc aag gcc agc atc cca ggg cca      348
Leu Leu Ile Gly Leu Leu Trp Ser Val Lys Ala Ser Ile Pro Gly Pro
45 50 55 60
cct cga tgg gac ccc tat cac ctc tcc aga gac ctg tac tac ctc act      396
Pro Arg Trp Asp Pro Tyr His Leu Ser Arg Asp Leu Tyr Tyr Leu Thr
65 70 75
gtg gag tcc tca gag aag gag agc tgc agg acc ccc aaa gtg gtt gac      444
Val Glu Ser Ser Glu Lys Glu Ser Cys Arg Thr Pro Lys Val Val Asp
80 85 90
atc ccc act tac gag gaa gcc gtg agc ttc cca gtg gcc gag ggg ccc      492
Ile Pro Thr Tyr Glu Glu Ala Val Ser Phe Pro Val Ala Glu Gly Pro
95 100 105
cca aca cca cct gca tac cct acg gag gaa gcc ctg gag cca agt gga      540
Pro Thr Pro Pro Ala Tyr Pro Thr Glu Glu Ala Leu Glu Pro Ser Gly
110 115 120
tcg agg gat gcc ctg ctc agc acc cag ccc gcc tgg cct cca ccc agc      588
Ser Arg Asp Ala Leu Leu Ser Thr Gln Pro Ala Trp Pro Pro Pro Ser
125 130 135 140
tat gag agc atc agc ctt gct ctt gat gcc gtt tct gca gag acg aca      636
Tyr Glu Ser Ile Ser Leu Ala Leu Asp Ala Val Ser Ala Glu Thr Thr
145 150 155
ccg agt gcc aca cgc tcc tgc tca ggc ctg gtt cag act gca cgg gga      684
Pro Ser Ala Thr Arg Ser Cys Ser Gly Leu Val Gln Thr Ala Arg Gly
160 165 170
gga agt taaaggctcc tagcaggtcc tgaatccaga gacaaaaatg ctgtgccttc      740
Gly Ser
tccagagtct tatgcagtgc ctgggacaca gtaggcactc agcaaacggt cggtgttgaa      800
ggctgttcta tttatctatt gctgtataac aaaccacccc agaatttagt ggcttaaaat      860
aaatcccatt ttattacgaa aaaaaaaaaa aaaa      894

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&lt;210&gt; 51

&lt;211&gt; 1447

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 501..1253

&lt;220&gt;



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<221> sig_peptide
<222> 501..1229
<223> Von Heijne matrix
      score 4.10
      seq LPSLAHLLPALDC/LE
```

```
<220>
<221> polyA_signal
<222> 1392..1397
```

```
<220>
<221> polyA_site
<222> 1432..1447
```

```
<220>  
<221> misc_feature  
<222> 243,252,278,285,387,1429  
<223> n=a, g, c or t
```

<400> 51															
gtgagtcagg	tggtctctgg	gcccaggaac	cggtggacgc	cctacagctg		60									
agaaggggac	ccaaggggtc	ggccgcggcc	aaggcccccta	ggaccgccgc	cccagctcac	120									
gctgccgacg	gcagctatag	acattctgcg	tcaggtcccg	gtctctggac	tttgcccttc	180									
ccgagccctg	gaggtgggga	gaaaagggtt	accaattttt	aaaatccaaa	tatatctcat	240									
ggntacagt	gnaagaactg	gccagagagt	ctggaagntt	tgggnttctg	gtcctggctg	300									
tgccactgac	tcactgtgac	cttgggatct	tgtgctgtga	agacatttcc	caagtgcctt	360									
atgttagcca	gcaaatctga	cccacanggc	ctggaaagag	gtgattgtta	ggttgccgag	420									
agggtggtctt	atccagctca	gcttccctctg	ggaccaccgc	tgggacctga	ggcagaactg	480									
gggtggactt	ggctctctcc	atg gca cac	cgg ctg cag	ata cga .ctg	ctg acg	533									
Met Ala His Arg Leu Gln Ile Arg Leu Leu Thr															
-240						-235									
tgg gat gtg aag gac acg ctg ctc agg ctc cgc cac ccc tta ggg gag		581													
Trp Asp Val Lys Asp Thr Leu Leu Arg Leu Arg His Pro Leu Gly Glu															
-230						-225									
-220															
gcc tat gcc acc aag gcc cgg gcc cat ggg ctg gag gtg gag ccc tca		629													
Ala Tyr Ala Thr Lys Ala Arg Ala His Gly Leu Glu Val Glu Pro Ser															
-215						-210									
-205															
gcc ctg gaa caa ggc ttc agg cag gca tac agg gct cag agc cac agc		677													
Ala Leu Glu Gln Gly Phe Arg Gln Ala Tyr Arg Ala Gln Ser His Ser															
-200						-195									
-190						-185									
ttc ccc aac tac ggc ctg agc cac ggc cta acc tcc cgc cag tgg tgg		725													
Phe Pro Asn Tyr Gly Leu Ser His Gly Leu Thr Ser Arg Gln Trp Trp															
-180						-175									
-170															
ctg gat gtg gtc ctg cag acc ttc cac ctg gcg ggt gtc cag gat gct		773													
Leu Asp Val Val Leu Gln Thr Phe His Leu Ala Gly Val Gln Asp Ala															
-165						-160									
-155															
cag gct gta gcc ccc atc gct gaa cag ctt tat aaa gac ttc agc cac		821													
Gln Ala Val Ala Pro Ile Ala Glu Gln Leu Tyr Lys Asp Phe Ser His															
-150						-145									
-140															
ccc tgc acc tgg cag gtg ttg gat ggg gct gag gac acc ctg agg gag		869													
Pro Cys Thr Trp Gln Val Leu Asp Gly Ala Glu Asp Thr Leu Arg Glu															
-135						-130									
-125															
tgc cgc aca cgg ggt ctg aga ctg gca gtg atc tcc aac ttt gac cga		917													
Cys Arg Thr Arg Gly Leu Arg Leu Ala Val Ile Ser Asn Phe Asp Arg															
-120						-115									
-110						-105									
cgg cta gag ggc atc ctg gag ggc ctt ggc ctg cgt gaa cac ttc gac		965													
Arg Leu Glu Gly Ile Leu Glu Gly Leu Gly Leu Arg Glu His Phe Asp															
-100						-95									
-90															
ttt gtg ctg acc tcc gag gct gct ggc tgg ccc aag ccg gac ccc cgc		1013													
Phe Val Leu Thr Ser Glu Ala Ala Gly Trp Pro Lys Pro Asp Pro Arg															
-85						-80									
-75															
att ttc cag gag gcc ttg cgg ctt gct cat atg gaa cca gta gtg gca		1061													

60

```

Ile Phe Gln Glu Ala Leu Arg Leu Ala His Met Glu Pro Val Val Ala
      -70                      -65                      -60
gcc cat gtt ggg gat aat tac ctc tgc gat tac cag ggg cct cgg gct      1109
Ala His Val Gly Asp Asn Tyr Leu Cys Asp Tyr Gln Gly Pro Arg Ala
      -55                      -50                      -45
gtg ggc atg cac agc ttc ctg gtg gtt ggc cca cag gca ctg gac ccc      1157
Val Gly Met His Ser Phe Leu Val Val Gly Pro Gln Ala Leu Asp Pro
      -40                      -35                      -30                      -25
gtg gtc agg gat tct gta cct aaa gaa cac atc ctc ccc tct ctg gcc      1205
Val Val Arg Asp Ser Val Pro Lys Glu His Ile Leu Pro Ser Leu Ala
      -20                      -15                      -10
cat ctc ctg cct gcc ctt gac tgc cta gag ggc tca act cca ggg ctt      1253
His Leu Leu Pro Ala Leu Asp Cys Leu Glu Gly Ser Thr Pro Gly Leu
      -5                      1                      5
tgaggccagt gagggaagtg gctgggccct aggccatgga gaaaacctta aacaaaccct      1313
ggagacaggg agcccccttct ttctccacag ctctggacct ttccccctct cctgcggcc      1373
tttgtcacct actgtgataa taaagcagtg agtgctgagc tctcaccctt ccccnccaa      1433
aaaaaaaaaa aaaa                                         1447

```

<210> 52  
 <211> 1540  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 25..402

<220>  
 <221> sig\_peptide  
 <222> 25..96  
 <223> Von Heijne matrix  
       score 7.00  
       seq LLCCFRALSGSLS/MR

<220>  
 <221> polyA\_signal  
 <222> 1500..1505

<220>  
 <221> polyA\_site  
 <222> 1525..1540

<220>  
 <221> misc\_feature  
 <222> 625,1411,1432,1440,1450,1506  
 <223> n=a, g, c or t

```

<400> 52
agcctggccc tccctctttc caaa atg gac aag tcc ctc ttg ctg gaa ctc      51
                        Met Asp Lys Ser Leu Leu Leu Glu Leu
                        -20
ccc atc ctg ctc tgc tgc ttt agg gca tta tct gga tca ctt tca atg      99
Pro Ile Leu Leu Cys Cys Phe Arg Ala Leu Ser Gly Ser Leu Ser Met
-15                      -10                      -5                      1
aga aat gat gca gtc aat gaa ata gtt gct gtg aaa aac aat ttt cct      147
Arg Asn Asp Ala Val Asn Glu Ile Val Ala Val Lys Asn Asn Phe Pro
      5                      10                      15
gtg ata gaa att att cag tgt agg atg tgc cac ctc cag ttc cca gga      195
Val Ile Glu Ile Ile Gln Cys Arg Met Cys His Leu Gln Phe Pro Gly
      20                      25                      30
gaa aag tgc tcc aga gga aga gga ata tgc aca gca aca aca gaa gag      243

```

61

Glu	Lys	Cys	Ser	Arg	Gly	Arg	Gly	Ile	Cys	Thr	Ala	Thr	Thr	Glu	Glu	
35					40						45					
gcc	tgc	atg	gtt	gga	agg	atg	ttc	aaa	agg	gat	ggg	aat	ccc	tgg	tta	291
Ala	Cys	Met	Val	Gly	Arg	Met	Phe	Lys	Arg	Asp	Gly	Asn	Pro	Trp	Leu	
50					55					60				65		
acc	ttc	atg	ggc	tgc	cta	aag	aac	tgt	gct	gat	gtg	aaa	ggc	ata	agg	339
Thr	Phe	Met	Gly	Cys	Leu	Lys	Asn	Cys	Ala	Asp	Val	Lys	Gly	Ile	Arg	
				70					75					80		
tgg	agt	gtc	tat	ttg	gtg	aac	ttc	agg	tgc	tgc	agg	agc	cat	gac	ctg	387
Trp	Ser	Val	Tyr	Leu	Val	Asn	Phe	Arg	Cys	Cys	Arg	Ser	His	Asp	Leu	
				85				90					95			
tgc	aat	gaa	gac	ctt	tagaag	ttaa	tggttcttct	gtgactccaa	tttctgggtg							442
Cys	Asn	Glu	Asp	Leu												
				100												
agg	tgt	gtc	ctc	agc	ctct	tcaca	atgac	tttctaaaaa	aaatcacaca	cacacacaca						502
cac	act	acag	aag	agg	attg	caa	acacatg	gctccatctt	ctgcacacga	aaggaaagtc						562
cct	ctc	cttt	tct	acagt	ct	gtcac	gcgc	ccttaaaata	agtaaataaa	taaccttgag						622
agn	aaaga	aac	aag	atca	ata	tat	cttgcag	gttgctacaa	acccttgtgc	tttctactgta						682
tag	ccag	ttc	att	cag	aaaa	gg	aggaaagg	gtagttaa	ttcaaaaaag	aatcccttcc						742
tct	ttct	ctct	gct	gctttc	ttc	cttctgt	ggcagggtat	tttaatat	ttttcaaatt							802
ttt	ttct	cttt	ctgt	gtttat	ctt	ctttat	cactccaaag	aaagcacata	actgtggcct							862
ga	aggg	atg	gg	agtag	caa	cata	aaaaaga	agtggctcaa	gtcttcttgg	agtttgttca						922
tga	atg	ctga	tccc	aggg	tg	agg	agaagat	tgggacatag	aaaggaaact	gcatcagaaa						982
cat	gaac	aga	gaa	agatt	gt	ctac	cttcta	gaatcagatc	tggttggggc	tgggggttgg						1042
aga	ataaaa	ag	cagg	aga	ag	ctat	gggatt	ctagaaatag	tacctgcac	cagcttccct						1102
gcc	aaact	ca	caagg	agaca	tca	acct	tcta	gacagggaac	agcttcagga	tacttccagg						1162
ag	acag	agcc	acc	agcag	ca	aa	caa	atat	tcccatgcct	ggagcatggc	atagaggaag					1222
ct	gag	aaatg	tgg	gggtctga	gga	agcc	att	tgagtctggc	cactagacat	ctcatcagcc						1282
act	tgt	gtga	ag	agatgccc	cat	gaccccc	gatgcctctc	ccacccttac	ctccatctca							1342
cac	act	tgag	ctt	gccactc	tgt	tata	aatc	ctctg	gagaaaaatg	gcagtttgac						1402
cga	acct	gnt	tc	acaagggt	ag	aggctgan	ttcta	acnga	aacttgtnag	aatgaagcct						1462
gg	aaag	agtg	at	gaattata	tt	attata	t	aaaaataat	aatnaaaaat	ataaagaaag						1522
ct	aaaaaaaa															1540

<210> 53  
 <211> 1643  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 280..678

<220>  
 <221> sig\_peptide  
 <222> 280..411  
 <223> Von Heijne matrix  
 score 3.90  
 seq LSDSLWSPHCSWS/ER

<220>  
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 <222> 1606..1611

<220>  
 <221> polyA\_site  
 <222> 1628..1643

<400> 53																
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cata	cattac	atttg	cttga	tgtct	ctcaa	ctgt	cttata	acct	ataaca	attg	ctccca					120
atcc	attttt	catg	ccatta	cttt	atttaa	aaacct	gggc	caac	ccagtt	ctca	aaaggt					180

62

```

attggacatc ctcagaaaag atgactgctc tatgttgaac caaacaactg attcttacag 240
gtttcttcct cacttgctct ctggctgtgg cagccagat atg gac agg aga gct 294
                                     Met Asp Arg Arg Ala
                                     -40
aca tcc ttc cct cca ctc cct gcc aaa gaa agg aga gct ggg ata agc 342
Thr Ser Phe Pro Pro Leu Pro Ala Lys Glu Arg Arg Ala Gly Ile Ser
                                     -35 -30 -25
agt gcc ctc ccc tgc cca ccc act atg tca ctt tct gac tcc ctt tgg 390
Ser Ala Leu Pro Cys Pro Pro Thr Met Ser Leu Ser Asp Ser Leu Trp
                                     -20 -15 -10
tcc cct cat tgc tct tgg agt gag aga cct cat tcc ttc tct cac tgg 438
Ser Pro His Cys Ser Trp Ser Glu Arg Pro His Ser Phe Ser His Trp
                                     -5 1 5
agg cag cca aga atg gga tcc tct ggt ggg tct ttg gat tat gta agt 486
Arg Gln Pro Arg Met Gly Ser Ser Gly Gly Ser Leu Asp Tyr Val Ser
10 15 20 25
ttc aaa cac tgg ata cac agc tcc aga tct aaa ggc aag att gct gct 534
Phe Lys His Trp Ile His Ser Ser Arg Ser Lys Gly Lys Ile Ala Ala
30 35 40
cta gag gca gga ctg ttc att tcc tgc ctt ggg gat gca ccc aga ggc 582
Leu Glu Ala Gly Leu Phe Ile Ser Cys Leu Gly Asp Ala Pro Arg Gly
45 50 55
ctg aat gct tcc caa gga aac caa aga aag aac atg gtc tgt ttc aga 630
Leu Asn Ala Ser Gln Gly Asn Gln Arg Lys Asn Met Val Cys Phe Arg
60 65 70
ggg gga gtg gcc agt cta gct ctg cca tct ctc act cct tcc tgc ctt 678
Gly Gly Val Ala Ser Leu Ala Leu Pro Ser Leu Thr Pro Ser Cys Leu
75 80 85
tagggtagca ctgaggtgga aagcctgaac tgctgtctct gctctggcct gtgctcaagc 738
tgtgtgtcct tggactggcc atctcctctc tgcaaccctc ggtcttctca tttgtaaaat 798
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cacagttggt ggtatttgag gagaaggtga agattttggt tggaagaggg accagcagac 978
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gggtctggaac tctgatctc aggtgatctg cctgcctcca cctcccaaag tgctggaatt 1458
acaggtgtga gctaccgctc cggccaatc tggggctcct agctttgggt caccaactac 1518
tcaaatcccc aacttctctc caagaggaat ttcaagaaac actgaccaat ctggttacag 1578
aagctgaagg ggccccaacc aggctgcaat aaacctgctt tacccttcca aaaaaaaaaa 1638
aaaaa 1643

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<210> 54  
 <211> 1314  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 64..726

<220>  
 <221> sig\_peptide  
 <222> 64..147  
 <223> Von Heijne matrix  
 score 3.70  
 seq VVFTLGMFSAGLS/DL

<220>  
 <221> polyA\_signal  
 <222> 1279..1284

<220>  
 <221> polyA\_site  
 <222> 1300..1314

<400> 54

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gta atg gag gcg ggc ggc ttt ctg gac tcg ctc att tac gga gca tgc      108
    Met Glu Ala Gly Gly Phe Leu Asp Ser Leu Ile Tyr Gly Ala Cys
          -25                      -20                      -15

gtg gtc ttc acc ctt ggc atg ttc tcc gcc ggc ctc tcg gac ctc agg      156
Val Val Phe Thr Leu Gly Met Phe Ser Ala Gly Leu Ser Asp Leu Arg
          -10                      -5                      1

cac atg cga atg acc cgg agt gtg gac aac gtc cag ttc ctg ccc ttt      204
His Met Arg Met Thr Arg Ser Val Asp Asn Val Gln Phe Leu Pro Phe
          5                      10                      15

ctc acc acg gaa gtc aac aac ctg ggc tgg ctg agt tat ggg gct ttg      252
Leu Thr Thr Glu Val Asn Asn Leu Gly Trp Leu Ser Tyr Gly Ala Leu
          20                      25                      30                      35

aag gga gac ggg atc ctc atc gtc gtc aac aca gtg ggt gct gcg ctt      300
Lys Gly Asp Gly Ile Leu Ile Val Val Asn Thr Val Gly Ala Ala Leu
          40                      45                      50

cag acc ctg tat atc ttg gca tat ctg cat tac tgc cct cgg aag cgt      348
Gln Thr Leu Tyr Ile Leu Ala Tyr Leu His Tyr Cys Pro Arg Lys Arg
          55                      60                      65

gtt gtg ctc cta cag act gca acc ctg cta ggg gtc ctt ctc ctg ggt      396
Val Val Leu Leu Gln Thr Ala Thr Leu Leu Gly Val Leu Leu Leu Gly
          70                      75                      80

tat ggc tac ttt tgg ctc ctg gta ccc aac cct gag gcc cgg ctt cag      444
Tyr Gly Tyr Phe Trp Leu Leu Val Pro Asn Pro Glu Ala Arg Leu Gln
          85                      90                      95

cag ttg ggc ctc ttc tgc agt gtc ttc acc atc agc atg tac ctc tca      492
Gln Leu Gly Leu Phe Cys Ser Val Phe Thr Ile Ser Met Tyr Leu Ser
          100                      105                      110                      115

cca ctg gct gac ttg gct aag gtg att caa act aaa tca acc caa tgt      540
Pro Leu Ala Asp Leu Ala Lys Val Ile Gln Thr Lys Ser Thr Gln Cys
          120                      125                      130

ctc tcc tac cca ctc acc att gct acc ctt ctc acc tct gcc tcc tgg      588
Leu Ser Tyr Pro Leu Thr Ile Ala Thr Leu Leu Thr Ser Ala Ser Trp
          135                      140                      145

tgc ctc tat ggg ttt cga ctc aga gat ccc tat atc atg gtg tcc aac      636
Cys Leu Tyr Gly Phe Arg Leu Arg Asp Pro Tyr Ile Met Val Ser Asn
          150                      155                      160

ttt cca gga atc gtc acc agc ttt atc cgc ttc tgg ctt ttc tgg aag      684
Phe Pro Gly Ile Val Thr Ser Phe Ile Arg Phe Trp Leu Phe Trp Lys
          165                      170                      175

tac ccc cag gag caa gac agg aac tac tgg ctc ctg caa acc      726
Tyr Pro Gln Glu Gln Asp Arg Asn Tyr Trp Leu Leu Gln Thr
          180                      185                      190

tgaggctgct catctgacca ctgggcacct tagtgccaac ctgaaccaa gagacctcct      786
tgtttcagct gggcctgctg tccagcttcc caggtgcagt ggggtgtggg aacaagagat      846
gactttgagg ataaaaggac caaagaaaaa gctttactta gatgattgat tggggcctag      906
gagatgaaat cactttttat ttttttagaga tttttttttt ttaatttttg aggttggggg      966
gcaatcttta gaatatgcct taaaaggccg ggcgcggtgg ctcacgcctg taatcccagc      1026
actttgggag gccaaagggtg gcggatcgcc tgagggtcagg agttcaagac caacctgact      1086
aacatgggtga aaccccatct ctactaaaaa tacaaaatta gccaggcatg atggcacatg      1146
cctgtaatcc cagatacttg ggaggctgag gcaggagaat tgcttgaacc caggaggtgg      1206
aggttgcagt gagctgagat cgtgccattg tgatatgaat atgccttata tgctgatatg      1266
aatatgcctt aaaataaagt gttccccacc cctaaaaaaa aaaaaaaaaa      1314

```

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<210> 55
<211> 2356
<212> DNA
<213> Homo Sapiens
```

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<220>  
<221> CDS  
<222> 42..1097
```

```
<220>
<221> sig_peptide
<222> 42..110
<223> Von Heijne matrix
      score 4.40
      seq QFILLGTTSVVTA/AL
```

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<220>
<221> polyA_signal
<222> 2323..2328
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<220>  
<221> polyA_site  
<222> 2341..2356
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<400> 55															
atccttggcg ccacagtcgg ccaccggggc tcgccgcgct c atg gag agc gga ggg															56
Met Glu Ser Gly Gly															
-20															
cgg ccc tcg ctg tgc cag ttc atc ctc ctg ggc acc acc tct gtg gtc	104														
Arg Pro Ser Leu Cys Gln Phe Ile Leu Leu Gly Thr Thr Ser Val Val															
-15 -10 -5															
acc gcc gcc ctg tac tcc gtg tac cgg cag aag gcc cgg gtc tcc caa	152														
Thr Ala Ala Leu Tyr Ser Val Tyr Arg Gln Lys Ala Arg Val Ser Gln															
1 5 10															
gag ctc aag gga gct aaa aaa gtt cat ttg ggt gaa gat tta aag agt	200														
Glu Leu Lys Gly Ala Lys Lys Val His Leu Gly Glu Asp Leu Lys Ser															
15 20 25 30															
att ctt tca gaa gct cca gga aaa tgc gtg cct tat gct gtt ata gaa	248														
Ile Leu Ser Glu Ala Pro Gly Lys Cys Val Pro Tyr Ala Val Ile Glu															
35 40 45															
gga gct gtg cgg tct gtt aaa gaa acg ctt aac agc cag ttt gtg gaa	296														
Gly Ala Val Arg Ser Val Lys Glu Thr Leu Asn Ser Gln Phe Val Glu															
50 55 60															
aac tgc aag ggg gta att cag cgg ctg aca ctt cag gag cac aag atg	344														
Asn Cys Lys Gly Val Ile Gln Cgg Leu Thr Leu Gln Glu His Lys Met															
65 70 75															
gtg tgg aat cga acc acc cac ctt tgg aat gat tgc tca aag atc att	392														
Val Trp Asn Arg Thr Thr His Leu Trp Asn Asp Cys Ser Lys Ile Ile															
80 85 90															
cat cag agg acc aac aca gtg ccc ttt gac ctg gtg ccc cac gag gat	440														
His Gln Arg Thr Asn Thr Val Pro Phe Asp Leu Val Pro His Glu Asp															
95 100 105 110															
ggc gtg gat gtg gct gtg cga gtg ctg aag ccc ctg gac tca gtg gat	488														
Gly Val Asp Val Ala Val Arg Val Leu Lys Pro Leu Asp Ser Val Asp															
115 120 125															
ctg ggt cta gag act gtg tat gag aag ttc cac ccc tcg att cag tcc	536														
Leu Gly Leu Glu Thr Val Tyr Glu Lys Phe His Pro Ser Ile Gln Ser															
130 135 140															
ttc acc gat gtc atc ggc cac tac atc agc ggt gag cgg ccc aaa ggc	584														
Phe Thr Asp Val Ile Gly His Tyr Ile Ser Gly Glu Arg Pro Lys Gly															
145 150 155															

65

```

atc caa gag acc gag gag atg ctg aag gtg ggg gcc acc ctc aca ggg      632
Ile Gln Glu Thr Glu Glu Met Leu Lys Val Gly Ala Thr Leu Thr Gly
      160                      165                      170
gtt ggc gaa ctg gtc ctg gac aac aac tct gtc cgc ctg cag ccg ccc      680
Val Gly Glu Leu Val Leu Asp Asn Asn Ser Val Arg Leu Gln Pro Pro
      175                      180                      185                      190
aaa caa ggc atg cag tac tat cta agc agc cag gac ttc gac agc ctg      728
Lys Gln Gly Met Gln Tyr Tyr Leu Ser Ser Ser Gln Asp Phe Asp Ser Leu
                      195                      200                      205
ctg cag agg cag gag tgc agc gtc agg ctc tgg aag gtg ctg gcg ctg      776
Leu Gln Arg Gln Glu Ser Ser Val Arg Leu Trp Lys Val Leu Ala Leu
                      210                      215                      220
gtt ttt ggc ttt gcc aca tgt gcc acc ctc ttc ttc att ctc cgg aag      824
Val Phe Gly Phe Ala Thr Cys Ala Thr Leu Phe Phe Ile Leu Arg Lys
                      225                      230                      235
cag tat ctg cag cgg cag gag cgc ctg cgc ctc aag cag atg cag gag      872
Gln Tyr Leu Gln Arg Gln Glu Arg Leu Arg Leu Lys Gln Met Gln Glu
                      240                      245                      250
gag ttc cag gag cat gag gcc cag ctg ctg agc cga gcc aag cct gag      920
Glu Phe Gln Glu His Glu Ala Gln Leu Leu Ser Arg Ala Lys Pro Glu
      255                      260                      265                      270
gac agg gag agt ctg aag agc gcc tgt gta gtg tgt ctg agc agc ttc      968
Asp Arg Glu Ser Leu Lys Ser Ala Cys Val Val Cys Leu Ser Ser Phe
                      275                      280                      285
aag tcc tgc gtc ttt ctg gag tgt ggg cac gtt tgt tcc tgc acc gag      1016
Lys Ser Cys Val Phe Leu Glu Cys Gly His Val Cys Ser Cys Thr Glu
                      290                      295                      300
tgc tac cgc gcc ttg cca gag ccc aag aag tgc cct atc tgc aga cag      1064
Cys Tyr Arg Ala Leu Pro Glu Pro Lys Lys Cys Pro Ile Cys Arg Gln
                      305                      310                      315
gcg atc acc cgg gtg ata ccc ctg tac aac agc taatagtttg gaagccgcac      1117
Ala Ile Thr Arg Val Ile Pro Leu Tyr Asn Ser
      320                      325
agcttgacct ggaagcacc ctgccccctt ttcagggtatt tttatctcga ggccttttga      1177
ggagcagtggt tgggggtagc tgtcacctcc aggtatgatt gagggaggaa ttgggtagaa      1237
actctccaga cccatgcctc caatggcagg atgctgcctt tcccacctga gaggggaccc      1297
tgtccatgtg cagcctcatc agagcctcac cctgggagga tgccgtggcg tctcctccca      1357
ggagccagat cagtgcgagt gtgactgaaa atgcctcatc acttaagcac caaagccagt      1417
gatcagcagc tcttctgttc ctgtgtcttc tgtttttttc tgggtgaatcg ttgcttgctg      1477
tggacttggt ggaggactca gaggggagga aaggctgggc cccgagtaca acggatgcct      1537
tgggtgctgc ctccgaagag actctgccgc agcttttctt ctttttcttc atgccccggg      1597
aaacagtctt tcttcagaat tgtcaggctg ggcagggtcaa cttgtgttcc tttccctca      1657
cctgcttgcc tccttaacgc ctgcacgtgt gtgtagagga caaaagaaag tgaagtcagc      1717
acatccgctt ctgcccagat ggtcggggcc cggggcaaca gattgaagag agatcatgtg      1777
aagggcagtt ggtcaggcag gcctcctggt ttcgccactg gccctgattt gaactcctgc      1837
cacttgggag agctcggggt ggtccctggt tttccctcct ggagaatgag gcgcagaggc      1897
ctcgctcct gaaggacgca gtgtggatgc cactggccta gtgtcctggc ctcacagctt      1957
ccttgcaagg ctgtcacaag gaaaagcagc cggctggcac cctgagcata tgcctcttg      2017
gggctccctc atccagcccg tcgcagcttt gacatcttgg tgtactcatg tcgcttctcc      2077
ttgtgttacc cctcccagt attaccattt gcccctcacc tgcccttggt gagcctttta      2137
gtgcaagaca gatggggctg ttttccccc cctctgagta gttggaggtc acatacacag      2197
ctcttttttt attgcccttt tctgcctctg aatgttcac tctcgtcctc ctttgtgcag      2257
gcgaggaagg ggtgccctca ggggccgaca ctagtatgat gcagtgtcca gtgtgaacag      2317
cagaaattaa acatgttgca accaaaaaaa aaaaaaaaaa      2356

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&lt;210&gt; 56

&lt;211&gt; 1701

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

<222> 245..1399

<220>

<221> sig\_peptide

<222> 245..796

<223> Von Heijne matrix

score 5.10

seq GWLPLLLLSLVA/TW

<220>

<221> polyA\_signal

<222> 1669..1674

<220>

<221> polyA\_site

<222> 1687..1701

<400> 56

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atccccgcga gtggcccggc gatgtcgctc gtgctgctaa gcctggccgc gctgtgcagg      60
agcgccgtac cccgagagcc gaccgttcaa tgtggctctg aaactgggcc atctccagag      120
tggatgctac aacatgatct aatcccgga gacttgaggg acctccgagt agaacctgtt      180
acaactagt ttgcaacagg ggactattca attttgatga atgtaagctg ggtactccgg      240
gcag atg tgg aca ttt tcc tac atc ggc ttc cct gta gag ctg aac aca      289
      Met Trp Thr Phe Ser Tyr Ile Gly Phe Pro Val Glu Leu Asn Thr
                -180                -175                -170
gtc tat ttc att ggg gcc cat aaa att cct aat gca aat atg aat gaa      337
Val Tyr Phe Ile Gly Ala His Lys Ile Pro Asn Ala Asn Met Asn Glu
                -165                -160                -155
gat ggc cct tcc atg tct gtg aat ttc acc tca cca ggc tgc cta gac      385
Asp Gly Pro Ser Met Ser Val Asn Phe Thr Ser Pro Gly Cys Leu Asp
                -150                -145                -140
cac ata atg aaa tat aaa aaa aag gtc aag gcc gga agc ctg tgg      433
His Ile Met Lys Tyr Lys Lys Lys Cys Val Lys Ala Gly Ser Leu Trp
                -135                -130                -125
gat ccg aac atc act gct tgt aag aag aat gag gag aca gta gaa gtg      481
Asp Pro Asn Ile Thr Ala Cys Lys Lys Asn Glu Glu Thr Val Glu Val
                -120                -115                -110
aac ttc aca acc act ccc ctg gga aac aga tac atg gct ctt atc caa      529
Asn Phe Thr Thr Thr Pro Leu Gly Asn Arg Tyr Met Ala Leu Ile Gln
                -105                -100                -95                -90
cac agc act atc atc ggg ttt tct cag gtg ttt gag cca cac cag aag      577
His Ser Thr Ile Ile Gly Phe Ser Gln Val Phe Glu Pro His Gln Lys
                -85                -80                -75
aaa caa acg cga gct tca gtg gtg att cca gtg act ggg gat agt gaa      625
Lys Gln Thr Arg Ala Ser Val Val Ile Pro Val Thr Gly Asp Ser Glu
                -70                -65                -60
ggg gct acg gtg cag ctg act cca tat ttt cct act tgt ggc agc gac      673
Gly Ala Thr Val Gln Leu Thr Pro Tyr Phe Pro Thr Cys Gly Ser Asp
                -55                -50                -45
tgc atc cga cat aaa gga aca gtt gtg ctc tgc cca caa aca ggc gtc      721
Cys Ile Arg His Lys Gly Thr Val Val Leu Cys Pro Gln Thr Gly Val
                -40                -35                -30
cct ttc cct ctg gat aac aac aaa agc aag ccg gga ggc tgg ctg cct      769
Pro Phe Pro Leu Asp Asn Asn Lys Ser Lys Pro Gly Gly Trp Leu Pro
                -25                -20                -15                -10
ctc ctc ctg ctg tct ctg ctg gtg gcc aca tgg gtg ctg gtg gca ggg      817
Leu Leu Leu Leu Ser Leu Leu Val Ala Thr Trp Val Leu Val Ala Gly
                -5                1                5
atc tat cta atg tgg agg cac gaa agg atc aag aag act tcc ttt tct      865
Ile Tyr Leu Met Trp Arg His Glu Arg Ile Lys Lys Thr Ser Phe Ser
                10                15                20
acc acc aca cta ctg ccc ccc att aag gtt ctt gtg gtt tac cca tct      913

```



67

```

Thr Thr Thr Leu Leu Pro Pro Ile Lys Val Leu Val Val Tyr Pro Ser
 25          30          35
gaa ata tgt ttc cat cac aca att tgt tac ttc act gaa ttt ctt caa      961
Glu Ile Cys Phe His His Thr Ile Cys Tyr Phe Thr Glu Phe Leu Gln
 40          45          50          55
aac cat tgc aga agt gag gtc atc ctt gaa aag tgg cag aaa aag aaa      1009
Asn His Cys Arg Ser Glu Val Ile Leu Glu Lys Trp Gln Lys Lys Lys
          60          65          70
ata gca gag atg ggt cca gtg cag tgg ctt gcc act caa aag aag gca      1057
Ile Ala Glu Met Gly Pro Val Gln Trp Leu Ala Thr Gln Lys Lys Ala
          75          80          85
gca gac aaa gtc gtc ttc ctt ctt tcc aat gac gtc aac agt gtg tgc      1105
Ala Asp Lys Val Val Phe Leu Leu Ser Asn Asp Val Asn Ser Val Cys
          90          95          100
gat ggt acc tgt ggc aag agc gag ggc agt ccc agt gag aac tct caa      1153
Asp Gly Thr Cys Gly Lys Ser Glu Gly Ser Pro Ser Glu Asn Ser Gln
          105          110          115
gac ctc ttc ccc ctt gcc ttt aac ctt ttc tgc agt gat cta aga agc      1201
Asp Leu Phe Pro Leu Ala Phe Asn Leu Phe Cys Ser Asp Leu Arg Ser
          120          125          130          135
cag att cat ctg cac aaa tac gtg gtg gtc tac ttt aga gag att gat      1249
Gln Ile His Leu His Lys Tyr Val Val Val Tyr Phe Arg Glu Ile Asp
          140          145          150
aca aaa gac gat tac aat gct ctc agt gtc tgc ccc aag tac cac ctc      1297
Thr Lys Asp Asp Tyr Asn Ala Leu Ser Val Cys Pro Lys Tyr His Leu
          155          160          165
atg aag gat gcc act gct ttc tgt gca gaa ctt ctc cat gtc aag cag      1345
Met Lys Asp Ala Thr Ala Phe Cys Ala Glu Leu Leu His Val Lys Gln
          170          175          180
cag gtg tca gca gga aaa aga tca caa gcc tgc cac gat ggc tgc tgc      1393
Gln Val Ser Ala Gly Lys Arg Ser Gln Ala Cys His Asp Gly Cys Cys
          185          190          195
tcc ttg tagccacccc atgagaagca agagacctta aaggcttcct atccaccaa      1449
Ser Leu
200
ttacagggaa aaaacgtgtg atgatacctga agcttactat gcagcctaca aacagcctta      1509
gtaattaaaa cattttatac caataaaatt ttcaaatt gctaactaat gtagcattaa      1569
ctaacgattg gaaactacat ttacaacttc aaagctgttt tatacataga aatcaattac      1629
agttttaatt gaaaactata accattttga taatgcaaca ataaagcatc ttcagccaaa      1689
aaaaaaaaaa aa      1701

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&lt;210&gt; 57

&lt;211&gt; 772

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 235..441

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 235..303

&lt;223&gt; Von Heijne matrix

score 5.30

seq LLLDVTVFIPALP/FS

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 758..772

&lt;220&gt;

68

&lt;221&gt; misc\_feature

&lt;222&gt; 573

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 57

```

aatacctggc aatctgttta agatcattga caggcctgag agttttccat acggcctgca      60
ccctaaccctc tgggaagaaa atatccacaa tgaaatttct acaagattag aggaaggaga      120
gaggcaacgg ggattccatt tctactagga gtatcaacct ctgagaggga tatatccatc      180
tctgtggatg tcatctgctc tgcagaaaac cctttcttgg aactaccagg aaac atg      237

```

Met

```

aat ctg atg tgg acc ctc ctc ctt ttc ctc ctt ttg gac gta act gtc      285
Asn Leu Met Trp Thr Leu Leu Leu Phe Leu Leu Leu Asp Val Thr Val

```

-20

-15

-10

```

ttc att cca gcc ctg ccc ttc tca aca cga cat ata gac aac ccc agg      333
Phe Ile Pro Ala Leu Pro Phe Ser Thr Arg His Ile Asp Asn Pro Arg

```

-5

1

5

10

```

tcg tgg gtc cct aga gga cac cac cga tac tgt gat gtg atg atg agg      381
Ser Trp Val Pro Arg Gly His His Arg Tyr Cys Asp Val Met Met Arg

```

15

20

25

```

cgc cgt tgg ctg atc tat agg ggt aaa tgc gag cag atc cac aca ttc      429
Arg Arg Trp Leu Ile Tyr Arg Gly Lys Cys Glu Gln Ile His Thr Phe

```

30

35

40

```

att cat aga atc tgaccacat agcagatttc tgcagaactc caccactgcc      481
Ile His Arg Ile

```

45

```

ctgtaccaac agcccctcca tgtgcagctg ccacaacagt actcatgatg tcaatgtcac      541
tgactgcttt gccagcacag ggacccgacc tnttactgct cactacaaa aataaggagt      601
ccaccaggcc catgcgagtg ggctgcaaga aggggggcac tgttcacctg gatggctagg      661
ttcctcctga caacggcacc tgaatgactt gcaccctacg ccttcaaata tgtgcagcac      721
tgtcaaggctc ttctttgtaa atgcttcgctc ctttgcaaaa aaaaaaaaaa a      772

```

&lt;210&gt; 58

&lt;211&gt; 987

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 88..411

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 88..234

&lt;223&gt; Von Heijne matrix

score 4.70

seq LLLVSTWSADLMS/YR

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 938..943

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 964..987

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 828,832

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 58

```

ttttttcttt gacatgttca gatgttggca aggctgaaaa ctgcagggga tctggttggtg      60

```

69

```

ataatccagg cctgaatata tacaaat atg aac aag acc cac aag gac tgc tca      114
                               Met Asn Lys Thr His Lys Asp Cys Ser
                               -45
tca ccc cag tat tcc att tac aac atc ctg aat gaa ctc ccg acc agg      162
Ser Pro Gln Tyr Ser Ile Tyr Asn Ile Leu Asn Glu Leu Pro Thr Arg
-40                               -35                               -30                               -25
cct ata att ctc tct tgc agc caa ata tcc tgc tta ctc ctg gta tct      210
Pro Ile Ile Leu Ser Cys Ser Gln Ile Ser Cys Leu Leu Leu Val Ser
                               -20                               -15                               -10
acc tgg tca gca gac ctc atg agt tat cgc cca gtg aca aaa cca tcc      258
Thr Trp Ser Ala Asp Leu Met Ser Tyr Arg Pro Val Thr Lys Pro Ser
                               -5                               1                               5
caa aga tgc acc agt cca gca caa agt atg act gtc aat ctc aca aaa      306
Gln Arg Cys Thr Ser Pro Ala Gln Ser Met Thr Val Asn Leu Thr Lys
10                               15                               20
gat gta ggg ttc tac gag gat act cag agt ata aga att acg cta agt      354
Asp Val Gly Phe Tyr Glu Asp Thr Gln Ser Ile Arg Ile Thr Leu Ser
25                               30                               35                               40
gaa ata agc caa gcc cag aaa gac aca tac ttt att att tca tgt atc      402
Glu Ile Ser Gln Ala Gln Lys Asp Thr Tyr Phe Ile Ile Ser Cys Ile
                               45                               50                               55
tgt gga atc taaaagagtc aaattcatgg cagcagggag agggctgaag      451
Cys Gly Ile
aagggggaga tgttgatcaa agtttctatg tatacaaaga ccaaaccatc acattatgcc      511
tcataaatat atacaattat tatttgctaa ttacaagtaa agcaatacaa gaagaaaaaa      571
aggaatcata agtaaattcca tgacaagtga aaacgcaatg gagagaaggg aatcaatgat      631
tgaagaagag aaaggacagt ggatttataa ctgcttcgaa agagtgattt gactggcaaa      691
ggactgggga gaggtccttt gggaaatgga caaaaccctc gaatggttag gaaagacaat      751
ctctttataa atgcggggca taagctgagc acaaggtgaa gtttggcatg tactgccgtg      811
ggatgttgta aaaattnatg ntcaaaaagca aagcaattct tggttcatct gtgttcactg      871
tgagactagc ctattattgg ggttaaactt ataaacaaac ttctgttcat catttttttt      931
ctccaaaata aagtgatcaa attgtcccac agaaaaaaa aaaaaaaaaa aaaaaa      987

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&lt;210&gt; 59

&lt;211&gt; 1324

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 129..452

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 129..212

&lt;223&gt; Von Heijne matrix

score 5.20

seq LDIVISFVGAVSS/ST

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1290..1295

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1309..1324

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 888,1080

&lt;223&gt; n=a, g, c or t

70

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<400> 59
gattttttttt acaagcaata gtttagtagt tcaacttttca ttaattatatt ctagtaatta      60
ctttcagtat  tgaaaatact tactgttaat attcatgtaa gtaacaaaca tttaaataag      120
aaaaataa atg tat ttt cat ttt cta ggt gcc gga gca att ctt att cct      170
      Met Tyr Phe His Phe Leu Gly Ala Gly Ala Ile Leu Ile Pro
      -25 -20 -15
cgt tta gac att gtg att tcc ttc gtt gga gct gtg agc agc agc aca      218
Arg Leu Asp Ile Val Ile Ser Phe Val Gly Ala Val Ser Ser Ser Thr
      -10 -5 1
ttg gcc cta atc ctg cca cct ttg gtt gaa att ctt aca ttt tcg aag      266
Leu Ala Leu Ile Leu Pro Pro Leu Val Glu Ile Leu Thr Phe Ser Lys
      5 10 15
gaa cat tat aat ata tgg atg gtc ctg aaa aat att tct ata gca ttc      314
Glu His Tyr Asn Ile Trp Met Val Leu Lys Asn Ile Ser Ile Ala Phe
      20 25 30
act gga gtt gtt ggc ttc tta tta ggt aca tat ata act gtt gaa gaa      362
Thr Gly Val Val Gly Phe Leu Leu Gly Thr Tyr Ile Thr Val Glu Glu
      35 40 45 50
att att tat cct act ccc aaa gtt gta gct ggc act cca cag agt cct      410
Ile Ile Tyr Pro Thr Pro Lys Val Val Ala Gly Thr Pro Gln Ser Pro
      55 60 65
ttt cta aat ttg aat tca aca tgc tta aca tct ggt ttg aaa      452
Phe Leu Asn Leu Asn Ser Thr Cys Leu Thr Ser Gly Leu Lys
      70 75 80
tagtaaaagc agaatcatga gtcttctatt tttgtcccat ttctgaaaat tatcaagata      512
actagtaaaa tacattgcta tatacataaa aatggtaaca aactctgttt tctttggcac      572
gatattaata ttttggaagt aatcataact ctttaccagt agtggtaaac ctatgaaaaa      632
tccttgcttt taagtgttag caatagtcca aaaaattaag ttctgaaaat tgaaaaaatt      692
aaaatgtaaa aaaattaaag aataaaaata cttctattat tcttttatct cagtaagaaa      752
taccttaacc aagatatctc tcttttatgc tactcttttg ccactcactt gagaacagaa      812
taggatttca acaataagag aataaaaata gaacatgtat aacaaaaagc tctctccaga      872
tcatccctgt gaatgnccaa agtaaaacttt atgtacagtg taaaaaaaaa aaaatctcag      932
ttatgttttt attagccaaa ttctaattgat tggctcctgg aagtatagaa aactcccatt      992
aacataatat aagcatcaga aaattgcaaa cactagaatt aattttacac tctaattggta      1052
gttgatcttc atagtcaaga ggcactgntc aagatcatga cttagtgttt caatgaaatt      1112
tgacaaggga ctttaaaact tatccagtgc aactcccttg tttttcgtca gaggaaaagg      1172
aggcctagaa aggttaagta acttggtcga gaccactcag ccttgagatc aagaaaacct      1232
aatcttctga ctcccaggcc aggatgtttt atttctcaca tcatgtccaa gaaaaagaat      1292
aaattatgtt cagctcaaaa aaaaaaaaaa aa      1324

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<210> 60
<211> 1918
<212> DNA
<213> Homo Sapiens

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<220>
<221> CDS
<222> 238..612

<220>
<221> sig_peptide
<222> 238..348
<223> Von Heijne matrix
      score 9.40
      seq LLCCVLSASQLSS/QD

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<220>
<221> polyA_signal
<222> 1885..1890

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<220>
<221> polyA_site

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&lt;222&gt; 1905..1918

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 945,1624

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 60

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aaaaatctaa gcgacttcga tgccaaggaa gttgtgtaaa tgtgcacgcg ctacaccaca      60
cccagggtgg aaaccacagt tgcagagtca ttaaacaatc aattgtttgt ttaacatctg      120
tgataggcag ctttccttct tttcaacagt gatacctacg aaaatcaaaa taaatgcaag      180
ctgaggtttt gtgctcactg aaagggtgtg caaccccgaga aggccgacac aaaaaaaa      237
atg gta tgt gaa gat gca ccg tct ttt caa atg gcc tgg gag agt caa      285
Met Val Cys Glu Asp Ala Pro Ser Phe Gln Met Ala Trp Glu Ser Gln
      -35                               -30                               -25
atg gcc tgg gag agg ggg cct gcc ctt ctc tgc tgt gtc ctt tcg gct      333
Met Ala Trp Glu Arg Gly Pro Ala Leu Leu Cys Cys Val Leu Ser Ala
      -20                               -15                               -10
tcc cag ttg agc tcc caa gac cag gac cca ctg ggg cat ata aaa tct      381
Ser Gln Leu Ser Ser Gln Asp Gln Asp Pro Leu Gly His Ile Lys Ser
      -5                               1                               5                               10
ctg ctg tat cct ttc ggc ttc cca gtt gag ctc cca aga cca gga ccc      429
Leu Leu Tyr Pro Phe Gly Phe Pro Val Glu Leu Pro Arg Pro Gly Pro
      15                               20                               25
act ggg gca tat aaa aaa gtc aaa aat caa aat caa aca aca agt tct      477
Thr Gly Ala Tyr Lys Lys Val Lys Asn Gln Asn Gln Thr Thr Ser Ser
      30                               35                               40
gag tta ctt agg aaa cag act tcg cat ttc aat cag aga ggc cac aga      525
Glu Leu Leu Arg Lys Gln Thr Ser His Phe Asn Gln Arg Gly His Arg
      45                               50                               55
gca agg tct aaa ctt ctg gct tct aga caa att cct gat aga aca ttt      573
Ala Arg Ser Lys Leu Leu Ala Ser Arg Gln Ile Pro Asp Arg Thr Phe
      60                               65                               70                               75
aaa tgt ggg aag tgg ctt ccc cag gtc cca tcc cct gtt tagggataga      622
Lys Cys Gly Lys Trp Leu Pro Gln Val Pro Ser Pro Val
      80                               85
gttgatatca tttttatagt tgccatgtat gcctctgcct gaattttttt aattgacttt      682
tgagcttttg agattgcacg agggagaaca aggcctttgc tgttgtggat aggaaagact      742
taacctaaaa ttaaacacagc aagaaagcat tagtaaaaaat ctaacaatat gaagggctct      802
tatgagtcac ttttttcaaa agatgaaaac tccagaaacg cacaggaacg aaatacctcc      862
cagaaacatg aagcaatcat cgaagactca ctggtaatat ttttaaaaag tatacagatc      922
aaagcaaaaa gaagccatgt gtnaacaaag agaaatgtgc aaatattttt taaggcagta      982
ttaagtgcaa gaggagtaac atgaaataaa cattctttca catggctact gggaatataa      1042
atttcgctcc agaaaggccg tagcagtttg acgatagggtg gcaaaacctt aagatttgtg      1102
actggggccc agaattttta tttctaggaa tgtatcctga ggaaattatc cgagatcccc      1162
acaaactgca atgttttagga attgtcctta tagcattgca tacacaagaa aaacagagaa      1222
aagcctgacg cctgtcagtg gaaaaggggt tcaatgaatt acggtgtgtc tgcattgaggc      1282
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taacactttg ggaggccaag gtgggaagat tgcctgagct caggagtttg agaccagcct      1402
gggcaacacg gtgaaacccc gtctctacta aaatacaaaa aattagccgg gcgtcgcagc      1462
atgcgcctgt agtcccagct gctcaggagg ctgaggcagg agaattgatt gaacccggga      1522
ggcagagggt gcactgagct gagattaagc caccgcactc cagcctgggc gacagagcaa      1582
gattccgttc ccaagaaaaa aaaattgttc aacaataagg gncaaaggga gagaatcata      1642
acatctgatt aaacagaaaa agcaagattt ttaaaactaa ctatataagg atgggtcccag      1702
ctgtgtcaaa aggaagcttg tttgtaatac gtgtgcataa aaattaaata gaggtgaaca      1762
caattatttt aaggcagtta aattatctct gtattgtgaa ctaagacttt ctagaatttt      1822
acttattcat tctgtactta aattttttct aatgaacaca tatacttttg taatcagaaa      1882
atattaaatg catgtatttt tcaaaaaaaaa aaaaaa      1918

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&lt;210&gt; 61

&lt;211&gt; 852

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 229..735

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 229..492

&lt;223&gt; Von Heijne matrix

score 6.70

seq VFALSSFLNKASA/VY

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 816..821

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 841..852

&lt;400&gt; 61

aatgactggc agtggcatca gcgatggcgg ctgcgtcggg gtcggttctg cagcgtgta	60
tcgtgtcgcc ggcagggagg catagcgccct ctctgatctt cctgcatggc tcaggtgatt	120
ctggacaagg attaagaatg tggatcaagc aggttttttaa atcaagattt aacattccaa	180
cacataaaaa ttatttatcc aacagctcct cccagatcat atactcct atg aaa gga	237
Met Lys Gly	
gga atc tcc aat gta tgg ttt gac aga ttt aaa ata acc aat gac tgc	285
Gly Ile Ser Asn Val Trp Phe Asp Arg Phe Lys Ile Thr Asn Asp Cys	
-85 -80 -75 -70	
cca gaa cac ctt gaa tca att gat gtc atg tgt caa gtg ctt act gat	333
Pro Glu His Leu Glu Ser Ile Asp Val Met Cys Gln Val Leu Thr Asp	
-65 -60 -55	
ttg att gat gaa gaa gta aaa agt ggc atc aag aag aac agg ata tta	381
Leu Ile Asp Glu Glu Val Lys Ser Gly Ile Lys Lys Asn Arg Ile Leu	
-50 -45 -40	
ata gga gga ttc tct atg gga gga tgc atg gca atg cat tta gca tat	429
Ile Gly Gly Phe Ser Met Gly Gly Cys Met Ala Met His Leu Ala Tyr	
-35 -30 -25	
aga aat cat caa gat gtg gca gga gta ttt gct ctt tct agt ttt ctg	477
Arg Asn His Gln Asp Val Ala Gly Val Phe Ala Leu Ser Ser Phe Leu	
-20 -15 -10	
aat aaa gca tct gct gtt tac cag gct ctt cag aag agt aat ggt gta	525
Asn Lys Ala Ser Ala Val Tyr Gln Ala Leu Gln Lys Ser Asn Gly Val	
-5 1 5 10	
ctt cct gaa tta ttt cag tgt cat ggt act gca gat gag tta gtt ctt	573
Leu Pro Glu Leu Phe Gln Cys His Gly Thr Ala Asp Glu Leu Val Leu	
15 20 25	
cat tct tgg gca gaa gag aca aac tca atg tta aaa tct cta gga gtg	621
His Ser Trp Ala Glu Glu Thr Asn Ser Met Leu Lys Ser Leu Gly Val	
30 35 40	
acc acg aag ttt cat agt ttt cca aat gtt tac cat gag cta agc aaa	669
Thr Thr Lys Phe His Ser Phe Pro Asn Val Tyr His Glu Leu Ser Lys	
45 50 55	
act gag tta gac ata ttg aag tta tgg att ctt aca aag ctg cca gga	717
Thr Glu Leu Asp Ile Leu Lys Leu Trp Ile Leu Thr Lys Leu Pro Gly	
60 65 70 75	
gaa atg gaa aaa caa aaa tgaatgaatc aagagtgatt tgttaatgta	765
Glu Met Glu Lys Gln Lys	
80	
agtgtaatgt ctttgtgaaa agtgattttt actgccaaat tataatgata attaaaatat	825
taagaaatag caaaaaaaaaa aaaaaaa	852

<210> 62  
 <211> 726  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 168..413

<220>  
 <221> sig\_peptide  
 <222> 168..335  
 <223> Von Heijne matrix  
 score 3.80  
 seq QMIMLVCFNLSRG/CL

<220>  
 <221> polyA\_signal  
 <222> 684..689

<220>  
 <221> polyA\_site  
 <222> 708..726

<220>  
 <221> misc\_feature  
 <222> 723  
 <223> n=a, g, c or t

<400> 62  
 cagcaaaatg gcagggaagg cagctctaag ctcccatcct tccataggaa tgttgaataa 60  
 acaaccagac actgtcagaa ccaactttgt gagaaccggg aaaataatca aagggtgtacg 120  
 gcaactaaaa gaatgctgga tcaacacaaa ggaaacttaa aaatgat atg aaa gct 176  
 Met Lys Ala  
 -55  
 gtg tgg cat ttt tgc ttg tcc cac aag tcc agc ttg gtg ata gtc ttg 224  
 Val Trp His Phe Cys Leu Ser His Lys Ser Ser Leu Val Ile Val Leu  
 -50 -45 -40  
 aag acg gca ggc tgg att ccc cag gct ggg acc ctt atc cct ggt tcc 272  
 Lys Thr Ala Gly Trp Ile Pro Gln Ala Gly Thr Leu Ile Pro Gly Ser  
 -35 -30 -25  
 aga gag gag agc aga tct gat tca caa atg att atg ctt gtc tgt ttt 320  
 Arg Glu Glu Ser Arg Ser Asp Ser Gln Met Ile Met Leu Val Cys Phe  
 -20 -15 -10  
 aat ctt tcc aga ggc tgt ctg aag aag gta ttc atc atc tct gtt tta 368  
 Asn Leu Ser Arg Gly Cys Leu Lys Lys Val Phe Ile Ile Ser Val Leu  
 -5 1 5 10  
 cct gac cca gaa acc att ctg cta gga aaa aca gtg ggc att gct 413  
 Pro Asp Pro Glu Thr Ile Leu Leu Gly Lys Thr Val Gly Ile Ala  
 15 20 25  
 tgaaaacagt gttctgtggt tgaaaaaccc acagtcacct tgggctggtg ggaatgtaaa 473  
 atggcgcttc ttctggatca tcgtttggca gtttctcaaa aggtcaaacg tagaatcact 533  
 atttgatcca acaattctac tcctaggat atccccaaaa gaattgaaaa caaggatgca 593  
 aacatatgcg tgtacactaa tgtttataga aaaaatattc acaataatca aaaggcagaa 653  
 acaaccaag tgtccaataa cagaagaatg aataaacagt gtgatataaa cataaaaaaa 713  
 aaaaaaaaaa aaa 726

<210> 63  
 <211> 1039  
 <212> DNA  
 <213> Homo Sapiens

74

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<220>  
<221> CDS  
<222> 100..852
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<220>
<221> sig_peptide
<222> 100..159
<223> Von Heijne matrix
      score 6.10
      seq FLILFLFLMECQL/HL
```

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<220>
<221> polyA_signal
<222> 998..1003
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<220>  
<221> polyA_site  
<222> 1019..1039
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<400> 63															
agaacttctt gattcctcag ataaatagag gacagatgct ggactgtagc taagtatttc															60
ctttcatcta cgggataaaa tactgataat ttgagagtg atg gac aag gtt cag															114
Met Asp Lys Val Gln															
-20															
agt ggt ttc ctc att ttg ttt ttg ttt tta atg gaa tgc caa ctt cat															162
Ser Gly Phe Leu Ile Leu Phe Leu Phe Leu Met Glu Cys Gln Leu His															
-15 -10 -5 1															
tta tgc ttg ccg tat gca gat gga ctc cat ccc act gga aac ata aca															210
Leu Cys Leu Pro Tyr Ala Asp Gly Leu His Pro Thr Gly Asn Ile Thr															
5 10 15															
ggc tta cca ggt agc ttc aac cac tgg ttt tat gtg act cag gga gaa															258
Gly Leu Pro Gly Ser Phe Asn His Trp Phe Tyr Val Thr Gln Gly Glu															
20 25 30															
ttg aaa agc tgt ttc agg gga gat aaa aag aag gta att aca ttt cac															306
Leu Lys Ser Cys Phe Arg Gly Asp Lys Lys Lys Val Ile Thr Phe His															
35 40 45															
cgc aaa aag ttt tct ttt caa ggc agt aaa cgg tca caa cca ccc aga															354
Arg Lys Lys Phe Ser Phe Gln Gly Ser Lys Arg Ser Gln Pro Pro Arg															
50 55 60 65															
aac atc acc aaa gag ccc aaa gtg ttc ttt cat aaa acc cag ttg cct															402
Asn Ile Thr Lys Glu Pro Lys Val Phe Phe His Lys Thr Gln Leu Pro															
70 75 80															
ggg att caa ggg gct gcc tcg aga tcc acg gct gca tcc cct acg aac															450
Gly Ile Gln Gly Ala Ala Ser Arg Ser Thr Ala Ala Ser Pro Thr Asn															
85 90 95															
ccc atg aaa ttc ctg agg aat aaa gca ata att cgg cat aga cct gct															498
Pro Met Lys Phe Leu Arg Asn Lys Ala Ile Ile Arg His Arg Pro Ala															
100 105 110															
ctt gtt aaa gta att tta att tcg agc gta gcc ttc agc att gcc ctg															546
Leu Val Lys Val Ile Leu Ile Ser Ser Val Ala Phe Ser Ile Ala Leu															
115 120 125															
ata tgt ggg atg gca atc tcc tat atg ata tat cga ctg gca cag gct															594
Ile Cys Gly Met Ala Ile Ser Tyr Met Ile Tyr Arg Leu Ala Gln Ala															
130 135 140 145															
gag gaa aga caa cag ctc gag tca ctt tat aag aac ctc agg ata ccg															642
Glu Glu Arg Gln Gln Leu Glu Ser Leu Tyr Lys Asn Leu Arg Ile Pro															
150 155 160															
tta tta gga gat gaa gaa gag ggc tca gag gac gag ggt gag tcc acg															690
Leu Leu Gly Asp Glu Glu Glu Gly Ser Glu Asp Glu Gly Ser Thr															
165 170 175															
cac cta ctt cca aag aac gaa aat gag ctg gaa aag ttc atc cac tca															738



75

```

His Leu Leu Pro Lys Asn Glu Asn Glu Leu Glu Lys Phe Ile His Ser
      180              185              190
gtt att ata tca aaa aga agc aaa aat att aag aag aaa ctg aag gaa      786
Val Ile Ile Ser Lys Arg Ser Lys Asn Ile Lys Lys Lys Leu Lys Glu
      195              200              205
gag caa aac tca gta aca gaa aac aaa aca aag aat gcg tca cat aat      834
Glu Gln Asn Ser Val Thr Glu Asn Lys Thr Lys Asn Ala Ser His Asn
      210              215              220              225
gga aaa atg gaa gac ttg tgaacgcaga cgacagaggt gccggctgag      882
Gly Lys Met Glu Asp Leu
      230
gcagaggaga aactatgggg gtgctgggag actgagcctg tgggcgtggc ttgctcccag      942
agaaccttat ggaagaggac atcaaagaaa gaaatgccag acctgtatcc cagaaaataa      1002
agccacatga tatagcaaaa aaaaaaaaaa aaaaaaa      1039

```

```

<210> 64
<211> 1355
<212> DNA
<213> Homo Sapiens

```

```

<220>
<221> CDS
<222> 238..1152

```

```

<220>
<221> sig_peptide
<222> 238..339
<223> Von Heijne matrix
      score 8.50
      seq SIFLLLSFPDSNG/KA

```

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<220>
<221> polyA_signal
<222> 1298..1303

```

```

<220>
<221> polyA_site
<222> 1324..1355

```

```

<400> 64
aattttcttg aaatcacatg gtaccaatca caagtcttgt tattttgttt cattatgaga      60
aagataatct actaaatatt aaaatactgg aaggagcaag atagctttga tccagggaga      120
ccttttccat ttatgtgctt tagtaatctg ccgccaacaa gctatcttct ttatgttctt      180
ctacaactga tgttggtttg tttttctcat tttgtctctt aatagacaaa tggagggc      237
atg agc ttc ctt aga att acc cct tcg acg cat agt tct gtt tca tct      285
Met Ser Phe Leu Arg Ile Thr Pro Ser Thr His Ser Ser Val Ser Ser
      -30              -25              -20
gga ctt ttg agg ctt agt atc ttt cta cta ctt agc ttt cct gac tca      333
Gly Leu Leu Arg Leu Ser Ile Phe Leu Leu Leu Ser Phe Pro Asp Ser
      -15              -10              -5
aac gga aaa gcc att tgg aca gct cac ctg aat ata aca ttt cag gtt      381
Asn Gly Lys Ala Ile Trp Thr Ala His Leu Asn Ile Thr Phe Gln Val
      1              5              10
gga aat gag atc aca tcg gaa tta gga gag agt gga gtg ttc ggg aat      429
Gly Asn Glu Ile Thr Ser Glu Leu Gly Glu Ser Gly Val Phe Gly Asn
      15              20              25              30
cat tct cct ctg gaa agg gtg tct ggt gtg gtg gca ctt cct gaa gaa      477
His Ser Pro Leu Glu Arg Val Ser Gly Val Val Ala Leu Pro Glu Glu
      35              40              45
tgg aat cag aat gcc tgt cat cct ttg acc aat ttc agc agg ccc aaa      525
Trp Asn Gln Asn Ala Cys His Pro Leu Thr Asn Phe Ser Arg Pro Lys
      50              55              60

```

76

```

cag gca gac tca tgg ctg gcc ctc atc gaa cgt gga ggc tgt act ttt      573
Gln Ala Asp Ser Trp Leu Ala Leu Ile Glu Arg Gly Gly Cys Thr Phe
      65              70              75
aca cat aaa atc aac gtg gca gca gag aag gga gca aat ggg gtg atc      621
Thr His Lys Ile Asn Val Ala Ala Glu Lys Gly Ala Asn Gly Val Ile
      80              85              90
atc tac aac tat caa ggt acg ggc agt aaa gta ttt ccc atg tct cac      669
Ile Tyr Asn Tyr Gln Gly Thr Gly Ser Lys Val Phe Pro Met Ser His
      95              100              105              110
cag ggg acg gaa aat ata gtc gcg gtg atg ata agc aac ctg aaa ggc      717
Gln Gly Thr Glu Asn Ile Val Ala Val Met Ile Ser Asn Leu Lys Gly
      115              120              125
atg gaa att ttg cac tgc att cag aaa gga gtc tat gtg aca gtc atc      765
Met Glu Ile Leu His Ser Ile Gln Lys Gly Val Tyr Val Thr Val Ile
      130              135              140
att gaa gtg ggg aga atg cac atg cag tgg gtg agc cat tac atc atg      813
Ile Glu Val Gly Arg Met His Met Gln Trp Val Ser His Tyr Ile Met
      145              150              155
tat cta ttt acc ttc ctg gct gcc aca att gcc tac ttt tac tta gat      861
Tyr Leu Phe Thr Phe Leu Ala Ala Thr Ile Ala Tyr Phe Tyr Leu Asp
      160              165              170
tgc gtc tgg aga ctt aca cct aga gtg ccc aat tct ttc acc agg agg      909
Cys Val Trp Arg Leu Thr Pro Arg Val Pro Asn Ser Phe Thr Arg Arg
      175              180              185              190
cga agt caa ata aag aca gat gtg aag aaa gct att gac cag ctt caa      957
Arg Ser Gln Ile Lys Thr Asp Val Lys Lys Ala Ile Asp Gln Leu Gln
      195              200              205
ctg cga gtt ctc aaa gaa ggg gat gag gaa tta gac cta aat gaa gac      1005
Leu Arg Val Leu Lys Glu Gly Asp Glu Glu Leu Asp Leu Asn Glu Asp
      210              215              220
aac tgt gtt gtt tgc ttt gac aca tac aaa ccc caa gat gta gta cgc      1053
Asn Cys Val Val Cys Phe Asp Thr Tyr Lys Pro Gln Asp Val Val Arg
      225              230              235
att tta act tgc aaa cat ttt ttc cat aag gca tgc att gac ccc tgg      1101
Ile Leu Thr Cys Lys His Phe Phe His Lys Ala Cys Ile Asp Pro Trp
      240              245              250
ctt tta gcc cat agg aca tgt ccc atg tgc aag tgt gac atc ctg aaa      1149
Leu Leu Ala His Arg Thr Cys Pro Met Cys Lys Cys Asp Ile Leu Lys
      255              260              265              270
act taagaaatct ggagaatttt ctgaagatgt aaccagatct ttccaaatac      1202
Thr
aaagattaga taaattgtct tattgtactt tatgtagaga gaaaatttca gcttctctac      1262
ccaagtatga acaaggtga aatttgtgtt ttaaaaaataa aactccttat catgcccagc      1322
taaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa      1355

```

&lt;210&gt; 65

&lt;211&gt; 572

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 187..369

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 187..312

&lt;223&gt; Von Heijne matrix

score 7.10

seq LLPCSSVLTCGQA/SQ

&lt;220&gt;

<221> polyA\_signal  
<222> 489..494

<220>  
<221> polyA\_site  
<222> 558..572

<220>  
<221> misc\_feature  
<222> 94,527,537..538  
<223> n=a, g, c or t

<400> 65  
cttcttcagt cagtggctgg ataatctaata tataatgtta taatccatca tttctctttt 60  
tgaacagtca atttagttta acatttgctt aacnagccat tatgtatgcc aggtaatgtg 120  
ctagatgctg gtggttcaaa gaaaggaacg atgtggacct gacctcaaag aaatccattg 180  
gagaat atg aca gat tta gat tta atg atc aac ttt act ttt cct ata 228  
Met Thr Asp Leu Asp Leu Met Ile Asn Phe Thr Phe Pro Ile  
-40 -35 -30  
cag tgg gtc aac caa aac cgc atg gcg tac tac tct ctg aag cct cta 276  
Gln Trp Val Asn Gln Asn Arg Met Ala Tyr Tyr Ser Leu Lys Pro Leu  
-25 -20 -15  
cta ccc tgc tcc tcc gtg ttg aca tgt ggt cag gca agc cag gac tta 324  
Leu Pro Cys Ser Ser Val Leu Thr Cys Gly Gln Ala Ser Gln Asp Leu  
-10 -5 1  
ctc aca tca gct aca tca gtt act ggg atg gag aaa att gaa gcc 369  
Leu Thr Ser Ala Thr Ser Val Thr Gly Met Glu Lys Ile Glu Ala  
5 10 15  
tagaaagatc aagaaacttt ctccaggcca taaatagagg aatcaggatt caaatcagat 429  
agaccccagg gcttgttctc ttcaacacca cattacccta cattattatt caattattaa 489  
ataaaacctt gcattagtgg catttccaaa tgcataanca aaaaaatnna aaaaaaagta 549  
acactggcaa aaaaaaaaaa aaa 572

<210> 66  
<211> 535  
<212> DNA  
<213> Homo Sapiens

<220>  
<221> CDS  
<222> 121..459

<220>  
<221> sig\_peptide  
<222> 121..165  
<223> Von Heijne matrix  
score 4.20  
seq FYLLASSILCAL/IV

<220>  
<221> polyA\_signal  
<222> 497..502

<220>  
<221> polyA\_site  
<222> 521..535

<220>  
<221> misc\_feature  
<222> 486,489  
<223> n=a, g, c or t

78

```

<400> 66
agttacacca ggcattcctgg cccaaagttt cccaaatcca ggcggctaga ggcccactgc      60
ttcccaacta ccagctgagg ggggtccgtcc cgagaaggga gaagaggccg aagaggaaac      120
atg aac ttc tat tta ctc cta gcg agc agc att ctg tgt gcc ttg att      168
Met Asn Phe Tyr Leu Leu Leu Ala Ser Ser Ile Leu Cys Ala Leu Ile
-15          -10          -5          1
gtc ttc tgg aaa tat cgc cgc ttt cag aga aac act ggc gaa atg tca      216
Val Phe Trp Lys Tyr Arg Arg Phe Gln Arg Asn Thr Gly Glu Met Ser
5          10          15
tca aat tca act gct ctt gca cta gtg aga ccc tct tct tct ggg tta      264
Ser Asn Ser Thr Ala Leu Ala Leu Val Arg Pro Ser Ser Ser Gly Leu
20          25          30
att aac agc aat aca gac aac aat ctt gca gtc tac gac ctc tct cgg      312
Ile Asn Ser Asn Thr Asp Asn Asn Leu Ala Val Tyr Asp Leu Ser Arg
35          40          45
gat att tta aat aat ttc cca cac tca ata gcc agg cag aag cga ata      360
Asp Ile Leu Asn Asn Phe Pro His Ser Ile Ala Arg Gln Lys Arg Ile
50          55          60          65
ttg gta aac ctc agt atg gtg gaa aac aag ctg gtt gaa ctg gaa cat      408
Leu Val Asn Leu Ser Met Val Glu Asn Lys Leu Val Glu Leu Glu His
70          75          80
act cta ctt agc aag ggt ttc aga ggt gca tca cct cac cgg aaa tcc      456
Thr Leu Leu Ser Lys Gly Phe Arg Gly Ala Ser Pro His Arg Lys Ser
85          90          95
acc taaaagcgta caggatgtaa tgccagnggn ggaaatcatt aaagacactt      509
Thr
tgagtagatt caaaaaaaaaa aaaaaa      535

<210> 67
<211> 572
<212> DNA
<213> Homo Sapiens

<220>
<221> CDS
<222> 34..336

<220>
<221> sig_peptide
<222> 34..123
<223> Von Heijne matrix
      score 7.80
      seq SVTLAQLQLVQQ/GQ

<220>
<221> polyA_signal
<222> 536..541

<220>
<221> polyA_site
<222> 556..572

<220>
<221> misc_feature
<222> 545
<223> n=a, g, c or t

<400> 67
gcattacacg ccggtcagga ttgcgcaccc gac atg gag cgt ccc cgc agt ccc      54
Met Glu Arg Pro Arg Ser Pro
-30          -25
caa tgc tcg gcc ccg gcc tot gcc tca gct tcg gtt acc ctg gcg cag      102

```

79

[illegible]

```
<210> 68
<211> 804
<212> DNA
<213> Homo Sapiens
```

```
<220>  
<221> CDS  
<222> 119..409
```

```
<220>
<221> sig_peptide
<222> 119..388
<223> Von Heijne matrix
      score 4.30
      seq TCLTACWTALCCC/CL
```

```
<220>
<221> polyA_signal
<222> 769..774
```

```
<220>
<221> polyA_site
<222> 789..804
```

```
<220>
<221> misc_feature
<222> 274
<223> n=a, g, c or t
```

```
<220>
<221> unsure
<222> -39
<223> Xaa = His,Gln
```

```

<400> 68
acttgctctg agacaggtgc ggcaagtcta ctgcgggctg gtccgggctc ctcagggttca      60
gacccgaccg ttatccagtc ggttcgtgga gaggagaggt gcactttaca ggtcccca      118
atg aac caa gag aac cct cca cca tat cca ggc cct ggt cca acg gcc      166
Met Asn Gln Glu Asn Pro Pro Pro Tyr Pro Gly Pro Gly Pro Thr Ala
-90                      -85                      -80                      -75

```

```

cca tac cca cct tat cca cca caa cca atg ggt cca gga cct atg ggg      214
Pro Tyr Pro Pro Tyr Pro Pro Gln Pro Met Gly Pro Gly Pro Met Gly
               -70               -65               -60
gga ccc tac cca cct cct caa ggg tac ccc tac caa gga tac cta cag      262
Gly Pro Tyr Pro Pro Pro Gln Gly Tyr Pro Tyr Gln Gly Tyr Leu Gln
               -55               -50               -45
tac ggc tgg can ggt gga cct cag gag cct cct aaa acc aca gtg tat      310
Tyr Gly Trp Xaa Gly Gly Pro Gln Glu Pro Pro Lys Thr Thr Val Tyr
               -40               -35               -30
gtg gta gaa gac caa aga aga gat gag cta gga cca tcc acc tgc ctc      358
Val Val Glu Asp Gln Arg Arg Asp Glu Leu Gly Pro Ser Thr Cys Leu
               -25               -20               -15
aca gcc tgc tgg acg gct ctc tgt tgc tgc tgc tgc tgc tgc tgc tgc      406
Thr Ala Cys Trp Thr Ala Leu Cys Cys Cys Cys Cys Cys Cys Cys Cys
-10               -5               1               5
acc tgaccagacc agcccagccg tctgtctctg ccagctctgc tgccacctct      459
Thr
gacaggtgtg cctgccccca tctcttctga ttgctgttaa caaatgacta gctttgcaca      519
gacacctcta ccttcagcac tatgggattc tagattaatg ggggttgcta ctgtttaatt      579
cagtgacttg atctttttta tgtccaaaat ccatttctta ttgatcttta aagatgtgct      639
aaatgacttt tttggccaaa ggcttagttg tgaaaaatat aattttttaa ttatacatc      699
aaggtagtgg ccaaattgtt cacatcaatc atggaatgat ttctctgcta acagccgcct      759
gtatgtttca ataaatttgt ccaaagctca aaaaaaaaaa aaaaa      804

<210> 69
<211> 629
<212> DNA
<213> Homo Sapiens

<220>
<221> CDS
<222> 232..534

<220>
<221> sig_peptide
<222> 232..306
<223> Von Heijne matrix
      score 3.70
      seq AKTCLVLCSRVLV/VI

<220>
<221> polyA_signal
<222> 595..600

<220>
<221> polyA_site
<222> 615..629

<400> 69
tatcactggt acgaaccaag gatttacaga tcaactggcaa aaattctgag aactttcaca      60
ccagtatact gtccaagccc attaagtggc atcacacctc tcttttatgt agctcagaca      120
agacagtcta atatcttcaa aatactactg caatatggaa tcttagaaag agaaaaaac      180
cctatcaaca ttgtcttaac aatagtactc tacccttcga gagtaagagt a atg gtt      237
                               Met Val
                               -25
gat cgt gaa ttg gct gac atc cat gaa gat gcc aaa aca tgt ttg gta      285
Asp Arg Glu Leu Ala Asp Ile His Glu Asp Ala Lys Thr Cys Leu Val
               -20               -15               -10
cta tgt tcc aga gtg ctt tct gtc att tca gtc aag gaa ata aag aca      333
Leu Cys Ser Arg Val Leu Ser Val Ile Ser Val Lys Glu Ile Lys Thr
               -5               1               5
cag ctg agt tta gga aga cat cca att att tca aat tgg ttt gat tac      381

```

81

Gln	Leu	Ser	Leu	Gly	Arg	His	Pro	Ile	Ile	Ser	Asn	Trp	Phe	Asp	Tyr		
10					15					20					25		
att	cct	tca	aca	aga	tac	aaa	gat	cca	tgt	gaa	cta	tta	cat	ctt	tgc	429	
Ile	Pro	Ser	Thr	Arg	Tyr	Lys	Asp	Pro	Cys	Glu	Leu	Leu	His	Leu	Cys		
			30					35					40				
aga	cta	acc	atc	agg	aat	caa	cta	tta	acc	aac	aat	atg	ctc	cca	gat	477	
Arg	Leu	Thr	Ile	Arg	Asn	Gln	Leu	Leu	Thr	Asn	Asn	Met	Leu	Pro	Asp		
			45				50					55					
gga	ata	ttt	tca	ctt	cta	att	cct	gct	cgt	cta	caa	aac	tat	ctg	aat	525	
Gly	Ile	Phe	Ser	Leu	Leu	Ile	Pro	Ala	Arg	Leu	Gln	Asn	Tyr	Leu	Asn		
		60				65				70							
tta	gaa	atc	taacatacgt	cagtgctcta	agttccttaa	caatgcttac										574	
Leu	Glu	Ile															
		75															
caatgtatgg	cttagaagtt	aataaaaatt	cacttcatgc	aaaaaaaaaa	aaaaa											629	

&lt;210&gt; 70

&lt;211&gt; 669

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 140..595

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 140..442

&lt;223&gt; Von Heijne matrix

score 4.10

seq VFMLIVSVLALIP/ET

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 630..635

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 655..669

&lt;400&gt; 70

gagcggaag ccgagctggg cgagaagtag gggagggcgg tgctccgccg cgggtggcgg 60

tgctatcgct tcgcagaacc tactcaggca gccagctgag aagagttgag ggattgctgc 120

tgctgggtct gcagacgcg atg gat aac gtg cag ccg aaa ata aaa cat cgc 172

Met Asp Asn Val Gln Pro Lys Ile Lys His Arg

-100

-95

ccc ttc tgc ttc agt gtg aaa ggc cac gtg aag atg ctg cgg ctg gca 220

Pro Phe Cys Phe Ser Val Lys Gly His Val Lys Met Leu Arg Leu Ala

-90

-85

-80

-75

cta act gtg aca tct atg acc ttt ttt atc atc gca caa gcc cct gaa 268

Leu Thr Val Thr Ser Met Thr Phe Phe Ile Ile Ala Gln Ala Pro Glu

-70

-65

-60

cca tat att gtt atc act gga ttt gaa gtc acc gtt atc tta ttt ttc 316

Pro Tyr Ile Val Ile Thr Gly Phe Glu Val Thr Val Ile Leu Phe Phe

-55

-50

-45

ata ctt tta tat gta ctc aga ctt gat cga tta atg aag tgg tta ttt 364

Ile Leu Leu Tyr Val Leu Arg Leu Asp Arg Leu Met Lys Trp Leu Phe

-40

-35

-30

tgg cct ttg ctt gat att atc aac tca ctg gta aca aca gta ttc atg 412

Trp Pro Leu Leu Asp Ile Ile Asn Ser Leu Val Thr Thr Val Phe Met

-25

-20

-15

ctc atc gta tct gtg ttg gca ctg ata cca gaa acc aca aca ttg aca 460

82

```

Leu Ile Val Ser Val Leu Ala Leu Ile Pro Glu Thr Thr Thr Leu Thr
-10          -5          1          5
ggt ggt gga ggg gtg ttt gca ctt gtg aca gca gta tgc tgt ctt gcc      508
Val Gly Gly Gly Val Phe Ala Leu Val Thr Ala Val Cys Cys Leu Ala
          10          15          20
gac ggg gcc ctt att tac cgg aag ctt ctg ttc aat ccc agc ggt cct      556
Asp Gly Ala Leu Ile Tyr Arg Lys Leu Leu Phe Asn Pro Ser Gly Pro
          25          30          35
tac cag aaa aag cct gtg cat gaa aaa aaa gaa gtt ttg taattttata      605
Tyr Gln Lys Lys Pro Val His Glu Lys Lys Glu Val Leu
          40          45          50
ttacttttta gtttgatact aagtattaaa catatttctg tattcttcca aaaaaaaaaa      665
aaaa                                              669

```

<210> 71  
 <211> 973  
 <212> DNA  
 <213> Homo Sapiens

<220>  
 <221> CDS  
 <222> 32..658  
  
 <220>  
 <221> sig\_peptide  
 <222> 32..289  
 <223> Von Heijne matrix  
       score 4.00  
       seq KLWKLLFLMKSQG/WI

<220>  
 <221> polyA\_signal  
 <222> 936..941

<220>  
 <221> polyA\_site  
 <222> 959..973

<220>  
 <221> misc\_feature  
 <222> 934  
 <223> n=a, g, c or t

```

<400> 71
agggagaggg atggctagtg aggtttagat c atg ttg agc cct acc ttt gtt      52
                               Met Leu Ser Pro Thr Phe Val
                               -85          -80
ttg tgg gat gtt gga tat ccc tta tac acc tat gga tcc atc tgc att      100
Leu Trp Asp Val Gly Tyr Pro Leu Tyr Thr Tyr Gly Ser Ile Cys Ile
          -75          -70          -65
att gca tta att att tgg caa gtg aaa aag agc tgc caa aaa tta agc      148
Ile Ala Leu Ile Ile Trp Gln Val Lys Lys Ser Cys Gln Lys Leu Ser
          -60          -55          -50
ttg gta cct aac agg agc tgt tgc cgg tgt cac cga aga gtc caa caa      196
Leu Val Pro Asn Arg Ser Cys Cys Arg Cys His Arg Arg Val Gln Gln
          -45          -40          -35
aag tct gga gat aga aca tca aga gct agg aga act tca cag gaa gaa      244
Lys Ser Gly Asp Arg Thr Ser Arg Ala Arg Arg Thr Ser Gln Glu Glu
          -30          -25          -20
gcc gag aag ttg tgg aag ctg ctg ttt ctc atg aaa agc cag ggc tgg      292
Ala Glu Lys Leu Trp Lys Leu Leu Phe Leu Met Lys Ser Gln Gly Trp
-15          -10          -5          1

```



83

```

att cct cag gaa gga agt gtg cgg cga atc ctg tgt gca gac ccc tgc      340
Ile Pro Gln Glu Gly Ser Val Arg Arg Ile Leu Cys Ala Asp Pro Cys
      5              10              15
tgc caa atc tgc aat gtt atg gct ctg gag att aag caa ttg ctg gca      388
Cys Gln Ile Cys Asn Val Met Ala Leu Glu Ile Lys Gln Leu Leu Ala
      20              25              30
gaa gct cca gaa gtt ggc ttg gat aac aag atg aag ctg ttt ctg cac      436
Glu Ala Pro Glu Val Gly Leu Asp Asn Lys Met Lys Leu Phe Leu His
      35              40              45
tgg att aac cct gaa atg aaa gat cga agg cat gag gaa tcc att ctc      484
Trp Ile Asn Pro Glu Met Lys Asp Arg Arg His Glu Glu Ser Ile Leu
      50              55              60              65
ctt tct aag gct gag aca gtg acc caa gac agg aca aaa aac att gag      532
Leu Ser Lys Ala Glu Thr Val Thr Gln Asp Arg Thr Lys Asn Ile Glu
      70              75              80
aag agt cca act gtc acc aaa gat cat gtg tgg gga gct aca aca cag      580
Lys Ser Pro Thr Val Thr Lys Asp His Val Trp Gly Ala Thr Thr Gln
      85              90              95
aag aca aca gag gac cct gag gct cag cct cct tct act gag gag gaa      628
Lys Thr Thr Glu Asp Pro Glu Ala Gln Pro Pro Ser Thr Glu Glu Glu
      100             105             110
ggc ctg atc ttc tgt gat gcc ccc agt gcc taaataatct gctctagcaa      678
Gly Leu Ile Phe Cys Asp Ala Pro Ser Ala
      115             120
cactcccttc agtccagcca atcctgggtc ctgtgccact cctacaaatg ctccaaactc      738
tgtcctcaaa tgacttgtgc cactcaacca ggaaatctat cccaggtota actcacctca      798
gcagaaggca ctgttttatg caagaatacc catcacaaga aaaaggagtt cataggttcc      858
tgaacctctg caatcccctg aaaaaggctt tcattgccat ttccattaac atgcaggtga      918
agcagggcat tctccnaaat atactttgta cctttaagct aaaaaaaaaa aaaaa      973

```

&lt;210&gt; 72

&lt;211&gt; 791

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 14..280

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 14..76

&lt;223&gt; Von Heijne matrix

score 9.50

seq ALVVLCAFQLVAA/LE

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 776..791

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; 607

&lt;223&gt; n=a, g, c or t

&lt;400&gt; 72

```

ataggcgcgc acc atg ggc tcc tgc tcc ggc cgc tgc gcg ctc gtc gtc      49
      Met Gly Ser Cys Ser Gly Arg Cys Ala Leu Val Val
      -20              -15              -10
ctc tgc gct ttt cag ctg gtc gcc gcc ctg gag agg cag gtg ttt gac      97
Leu Cys Ala Phe Gln Leu Val Ala Ala Leu Glu Arg Gln Val Phe Asp
      -5              1              5

```

84

```

ttc ctg ggc tac cag tgg gcg ccc atc ctg gcc aac ttt gtc cac atc      145
Phe Leu Gly Tyr Gln Trp Ala Pro Ile Leu Ala Asn Phe Val His Ile
      10      15      20
atc atc gtc atc ctg gga ctc ttc ggc acc atc cag tac cgg ctg cgc      193
Ile Ile Val Ile Leu Gly Leu Phe Gly Thr Ile Gln Tyr Arg Leu Arg
      25      30      35
tat gtc atg tgt aca cgc tgt ggg cag ccg tct ggg tca cct gga acg      241
Tyr Val Met Cys Thr Arg Cys Gly Gln Pro Ser Gly Ser Pro Gly Thr
      40      45      50      55
tct tca tca tct gct tct acc tgg aag tcg gtg gcc tct taaaggacag      290
Ser Ser Ser Ser Ala Ser Thr Trp Lys Ser Val Ala Ser
      60      65
cgagctactg accttcagcc tctcccggca tcgctcctgg tggcgtgagc gctggccagg      350
ctgtctgcat gaggaggtgc cagcagtggg cctcggggcc ccccatggcc aggccctggt      410
gtcagggtgct ggctgtgcca tggagcccag ctatgtggag gccctacaca gttgcctgca      470
gatcctgata gcgcttctgg gctttgtctg tggctgccag gtggtcagcg tgtttacgga      530
ggaagaggac agctgcctgc gtaagtgagg aaacagctga tcctgctcct gtggcctcca      590
gcctcagcga ccgaccnagt gacaatgaca ggagctccca ggccctggga cgcgccccca      650
cccagacccc cccaggcggc cggcagcacc tgccctgggt tctaagtact ggacaccagc      710
cagggcgcca gggcagtgcc acggctggct gcagcgtcaa gagagtttgt aatttccttt      770
ctcttaaaaa aaaaaaaaaa a      791

```

&lt;210&gt; 73

&lt;211&gt; 1110

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 93..290

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; 93..149

&lt;223&gt; Von Heijne matrix

score 9.30

seq VFVFLFLWDPVLA/GI

&lt;220&gt;

&lt;221&gt; polyA\_signal

&lt;222&gt; 1078..1083

&lt;220&gt;

&lt;221&gt; polyA\_site

&lt;222&gt; 1096..1110

&lt;400&gt; 73

```

agtataggac tgtgtgctca acctcttctc tctgttcctt gacagccgat gtcagacctt      60
gccactagcc tccttaacag aagttcccag cc atg aag cct ctc ctt gtt gtg      113
                               Met Lys Pro Leu Leu Val Val
                               -15
ttt gtc ttt ctt ttc ctt tgg gat cca gtg ctg gca ggt ata aat tca      161
Phe Val Phe Leu Phe Leu Trp Asp Pro Val Leu Ala Gly Ile Asn Ser
      -10      -5      1
tta tca tca gaa atg cac aag aaa tgc tat aaa aat ggc atc tgc aga      209
Leu Ser Ser Glu Met His Lys Lys Cys Tyr Lys Asn Gly Ile Cys Arg
      5      10      15      20
ctt gaa tgc tat gag agt gaa atg tta gtt gcc tac tgt atg ttt cag      257
Leu Glu Cys Tyr Glu Ser Glu Met Leu Val Ala Tyr Cys Met Phe Gln
      25      30      35
ctg gag tgc tgt gtc aaa gga aat cct gca ccc tgacataaga aaccaatgaa      310
Leu Glu Cys Cys Val Lys Gly Asn Pro Ala Pro

```

```

      40      45
tggccactat cctgtaggcc cttgattctg ccattctttca caaaaccagg gaatttagat 370
caaactgtga caccatgatg tgtccatgac tactgggtttt tagcattttt ataggccagc 430
agactcttgt ggtcttaaat ttaaagagct gagctgtagc cttctttaaa agagctcgg 490
ttttcacaaa aacaatgtag aagatatttt ctcacctcaa cgtgatgtcc agtgtgtctca 550
tcagcacctg tttctccctc taatcataga ggatattctt attattttaga aaggcttcaa 610
gggaaacaac ttttgacacc taagtcgtgt cctaccttcg cttcagcttc gcatttccca 670
tttctgtgaa attccaaca gagaagcaga tttgccatgg cttcttgaca acctgtgtaca 730
tctctcacat aaaccgcata ggcagggcct gactacaggc tggcccagat ctgcactgag 790
tctgaccctg aagttccttt ggaacaggag aggccatctt gtgatgggct ggaacaaggt 850
aatttctcat ccacctccct agtttcagtt gagcaatgga actcccacc tgagccccta 910
gggttcagct acaggctata agactgccgt cctgtgggtt agtgttggtt ccttagcagc 970
agagtgatgc cacctctgct gcccgtcatc tgactcctct ggatgggtgt tatcctgtgg 1030
cttaagagct aacaccatgc tgatcttgct ttgctatatg tgtaactaat aaactgccta 1090
aatccaaaaa aaaaaaaaaa 1110

```

<210> 74  
 <211> 325  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -26...-1

<400> 74

```

Met Ala Thr Pro Leu Pro Pro Pro Ser Pro Arg His Leu Arg Leu Leu
-25 -20 -15
Arg Leu Leu Leu Ser Gly Leu Val Leu Gly Ala Ala Leu Arg Gly Ala
-10 -5 1 5
Ala Ala Gly His Pro Asp Val Ala Ala Cys Pro Gly Ser Leu Asp Cys
10 15 20
Ala Leu Lys Arg Arg Ala Arg Cys Pro Pro Gly Ala His Ala Cys Gly
25 30 35
Pro Cys Leu Gln Pro Phe Gln Glu Asp Gln Gln Gly Leu Cys Val Pro
40 45 50
Arg Met Arg Arg Pro Pro Gly Gly Gly Arg Pro Gln Pro Arg Leu Glu
55 60 65 70
Asp Glu Ile Asp Phe Leu Ala Gln Glu Leu Ala Arg Lys Glu Ser Gly
75 80 85
His Ser Thr Pro Pro Leu Pro Lys Asp Arg Gln Arg Leu Pro Glu Pro
90 95 100
Ala Thr Leu Gly Phe Ser Ala Arg Gly Gln Gly Leu Glu Leu Gly Leu
105 110 115
Pro Ser Thr Pro Gly Thr Pro Thr Pro His Thr Ser Leu Gly
120 125 130
Ser Pro Val Ser Ser Asp Pro Val His Met Ser Pro Leu Glu Pro Arg
135 140 145 150
Gly Gly Gln Gly Asp Gly Leu Ala Leu Val Leu Ile Leu Ala Phe Cys
155 160 165
Val Ala Gly Ala Ala Ala Leu Ser Val Ala Ser Leu Cys Trp Cys Arg
170 175 180
Leu Gln Arg Glu Ile Arg Leu Thr Gln Lys Ala Asp Tyr Ala Thr Ala
185 190 195
Lys Ala Pro Gly Ser Pro Ala Ala Pro Arg Ile Ser Pro Gly Asp Gln
200 205 210
Arg Leu Ala Gln Ser Ala Glu Met Tyr His Tyr Gln His Gln Arg Gln
215 220 225 230
Gln Met Leu Cys Leu Glu Arg His Lys Glu Pro Pro Lys Glu Leu Asp
235 240 245
Thr Ala Ser Ser Asp Glu Glu Asn Glu Asp Gly Asp Phe Thr Val Tyr
250 255 260

```

86

Glu Cys Pro Gly Leu Ala Pro Thr Gly Glu Met Glu Val Arg Asn Pro  
 265 270 275  
 Leu Phe Asp His Ala Ala Leu Ser Ala Pro Leu Pro Ala Pro Ser Ser  
 280 285 290  
 Pro Pro Ala Leu Pro  
 295

<210> 75  
 <211> 302  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -18..-1

<400> 75  
 Met Lys Ala Pro Gly Arg Leu Val Leu Ile Ile Leu Cys Ser Val Val  
 -15 -10 -5  
 Phe Ser Ala Val Tyr Ile Leu Leu Cys Cys Trp Ala Gly Leu Pro Leu  
 1 5 10  
 Cys Leu Ala Thr Cys Leu Asp His His Phe Pro Thr Gly Ser Arg Pro  
 15 20 25 30  
 Thr Val Pro Gly Pro Leu His Phe Ser Gly Tyr Ser Ser Val Pro Asp  
 35 40 45  
 Gly Lys Pro Leu Val Arg Glu Pro Cys Arg Ser Cys Ala Val Val Ser  
 50 55 60  
 Ser Ser Gly Gln Met Leu Gly Ser Gly Leu Gly Ala Glu Ile Asp Ser  
 65 70 75  
 Ala Glu Cys Val Phe Arg Met Asn Gln Ala Pro Thr Val Gly Phe Glu  
 80 85 90  
 Ala Asp Val Gly Gln Arg Ser Thr Leu Arg Val Val Ser His Thr Ser  
 95 100 105 110  
 Val Pro Leu Leu Leu Arg Asn Tyr Ser His Tyr Phe Gln Lys Ala Arg  
 115 120 125  
 Asp Thr Leu Tyr Met Val Trp Gly Gln Gly Arg His Met Asp Arg Val  
 130 135 140  
 Leu Gly Gly Arg Thr Tyr Arg Thr Leu Leu Gln Leu Thr Arg Met Tyr  
 145 150 155  
 Pro Gly Leu Gln Val Tyr Thr Phe Thr Glu Arg Met Met Ala Tyr Cys  
 160 165 170  
 Asp Gln Ile Phe Gln Asp Glu Thr Gly Lys Asn Arg Arg Gln Ser Gly  
 175 180 185 190  
 Ser Phe Leu Ser Thr Gly Trp Phe Thr Met Ile Leu Ala Leu Glu Leu  
 195 200 205  
 Cys Glu Glu Ile Val Val Tyr Gly Met Val Ser Asp Ser Tyr Cys Arg  
 210 215 220  
 Glu Lys Ser His Pro Ser Val Pro Tyr His Tyr Phe Glu Lys Gly Arg  
 225 230 235  
 Leu Asp Glu Cys Gln Met Tyr Leu Ala His Glu Gln Ala Pro Arg Ser  
 240 245 250  
 Ala His Arg Phe Ile Thr Glu Lys Ala Val Phe Ser Arg Trp Ala Lys  
 255 260 265 270  
 Lys Arg Pro Ile Val Phe Ala His Pro Ser Trp Arg Thr Glu  
 275 280

<210> 76  
 <211> 249  
 <212> PRT  
 <213> Homo Sapiens

<220>

87

<221> SIGNAL  
 <222> -15..-1

<400> 76

```

Met Leu Gln Leu Trp Lys Leu Val Leu Leu Cys Gly Val Leu Thr Gly
-15          -10          -5          1
Thr Ser Glu Ser Leu Leu Asp Asn Leu Gly Asn Asp Leu Ser Asn Val
      5          10          15
Val Asp Lys Leu Glu Pro Val Leu His Glu Gly Leu Glu Thr Val Asp
      20          25          30
Asn Thr Leu Lys Gly Ile Leu Glu Lys Leu Lys Val Asp Leu Gly Val
      35          40          45
Leu Gln Lys Ser Ser Ala Trp Gln Leu Ala Lys Gln Lys Ala Gln Glu
50          55          60          65
Ala Glu Lys Leu Leu Asn Asn Val Ile Ser Lys Leu Leu Pro Thr Asn
      70          75          80
Thr Asp Ile Phe Gly Leu Lys Ile Ser Asn Ser Leu Ile Leu Asp Val
      85          90          95
Lys Ala Glu Pro Ile Asp Asp Gly Lys Gly Leu Asn Leu Ser Phe Pro
      100          105          110
Val Thr Ala Asn Val Thr Val Ala Gly Pro Ile Ile Gly Gln Ile Ile
      115          120          125
Asn Leu Lys Ala Ser Leu Asp Leu Leu Thr Ala Val Thr Ile Glu Thr
130          135          140          145
Asp Pro Gln Thr His Gln Pro Val Ala Val Leu Gly Glu Cys Ala Ser
      150          155          160
Asp Pro Thr Ser Ile Ser Leu Ser Leu Leu Asp Lys His Ser Gln Ile
      165          170          175
Ile Asn Lys Phe Val Asn Ser Val Ile Asn Thr Leu Lys Ser Thr Val
      180          185          190
Ser Ser Leu Leu Gln Lys Glu Ile Cys Pro Leu Ile Arg Ile Phe Ile
      195          200          205
His Ser Leu Asp Val Asn Val Ile Gln Gln Val Val Asp Asn Pro Gln
210          215          220          225
His Lys Thr Gln Leu Gln Thr Leu Ile
      230

```

<210> 77  
 <211> 84  
 <212> PRT  
 <213> Homo Sapiens

<400> 77

```

Met Lys Val Lys Ile Lys Cys Trp Asn Gly Val Ala Thr Trp Leu Trp
1          5          10          15
Val Ala Asn Asp Glu Asn Cys Gly Ile Cys Arg Met Ala Phe Asn Gly
      20          25          30
Cys Cys Pro Asp Cys Lys Val Pro Gly Asp Asp Cys Pro Leu Val Trp
      35          40          45
Gly Gln Cys Ser His Cys Phe His Met His Cys Ile Leu Lys Trp Leu
      50          55          60
His Ala Gln Gln Val Gln Gln His Cys Pro Met Cys Arg Gln Glu Trp
      65          70          75          80
Lys Phe Lys Glu

```

<210> 78  
 <211> 554  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL

<222> -13...-1

**<220>**

<221> UNSURE

**<222> 259**

<223> Xaa = Asp, His, Asn, Tyr

<400> 78

Met	Leu	Tyr	Leu	Gln	Gly	Trp	Ser	Met	Pro	Ala	Val	Ala	Glu	Val	Lys
			-10					-5					1		
Leu	Arg	Asp	Asp	Gln	Tyr	Thr	Leu	Glu	His	Met	His	Ala	Phe	Gly	Met
5						10					15				
Tyr	Asn	Tyr	Leu	His	Cys	Asp	Ser	Trp	Tyr	Gln	Asp	Ser	Val	Tyr	Tyr
20					25					30					35
Ile	Asp	Thr	Leu	Gly	Arg	Ile	Met	Asn	Leu	Thr	Val	Met	Leu	Asp	Thr
				40					45					50	
Ala	Leu	Gly	Lys	Pro	Arg	Glu	Val	Phe	Arg	Leu	Pro	Thr	Asp	Leu	Thr
			55					60					65		
Ala	Cys	Asp	Asn	Arg	Leu	Cys	Ala	Ser	Ile	His	Phe	Ser	Ser	Ser	Thr
			70				75					80			
Trp	Val	Thr	Leu	Ser	Asp	Gly	Thr	Gly	Arg	Leu	Tyr	Val	Ile	Gly	Thr
	85					90					95				
Gly	Glu	Arg	Gly	Asn	Ser	Ala	Ser	Glu	Lys	Trp	Glu	Ile	Met	Phe	Asn
100					105					110					115
Glu	Glu	Leu	Gly	Asp	Pro	Phe	Ile	Ile	Ile	His	Ser	Ile	Ser	Leu	Leu
				120					125					130	
Asn	Ala	Glu	Glu	His	Ser	Ile	Ala	Thr	Leu	Leu	Leu	Arg	Ile	Glu	Lys
				135				140					145		
Glu	Glu	Leu	Asp	Met	Lys	Gly	Ser	Gly	Phe	Tyr	Val	Ser	Leu	Glu	Trp
			150				155					160			
Val	Thr	Ile	Ser	Lys	Lys	Asn	Gln	Asp	Asn	Lys	Lys	Tyr	Glu	Ile	Ile
						170					175				
Lys	Arg	Asp	Ile	Leu	Arg	Gly	Lys	Ser	Val	Pro	His	Tyr	Ala	Ala	Ile
180					185					190					195
Lys	Pro	Asp	Gly	Asn	Gly	Leu	Met	Ile	Val	Ser	Tyr	Lys	Ser	Leu	Thr
				200					205					210	
Phe	Val	Gln	Ala	Gly	Gln	Asp	Leu	Glu	Glu	Asn	Met	Asp	Glu	Asp	Ile
				215				220					225		
Ser	Glu	Lys	Ile	Lys	Glu	Pro	Leu	Tyr	Tyr	Trp	Gln	Gln	Thr	Glu	Asp
							235					240			
Asp	Leu	Thr	Val	Thr	Ile	Arg	Leu	Pro	Glu	Asp	Ser	Thr	Lys	Glu	Xaa
						250					255				
Ile	Gln	Ile	Gln	Phe	Leu	Pro	Asp	His	Ile	Asn	Ile	Val	Leu	Lys	Asp
260					265					270					275
His	Gln	Phe	Leu	Glu	Gly	Lys	Leu	Tyr	Ser	Ser	Ile	Asp	His	Glu	Ser
				280					285					290	
Ser	Thr	Trp	Ile	Ile	Lys	Glu	Ser	Asn	Ser	Leu	Glu	Ile	Ser	Leu	Ile
								300					305		
Lys	Lys	Asn	Glu	Gly	Leu	Thr	Trp	Pro	Glu	Leu	Val	Ile	Gly	Asp	Lys
							315					320			
Gln	Gly	Glu	Leu	Ile	Arg	Asp	Ser	Ala	Gln	Cys	Ala	Ala	Ile	Ala	Glu
						330					335				
Arg	Leu	Met	His	Leu	Thr	Ser	Glu	Glu	Leu						

89

Asp Ala Leu Leu Trp Gln Pro His Ser Ser Lys Gln Asp Asp Met Trp  
 420 425 430 435  
 Glu His Ile Ala Thr Phe Asn Ala Leu Gly Tyr Val Gln Ala Ser Lys  
 440 445 450  
 Arg Asp Lys Lys Phe Phe Ala Cys Ala Pro Asn Tyr Ser Tyr Ala Ala  
 455 460 465  
 Leu Cys Glu Cys Leu Arg Arg Val Phe Ile Tyr Arg Gln Pro Ala Pro  
 470 475 480  
 Met Ser Thr Val Leu Tyr Asn Arg Lys Glu Gly Arg Gln Val Gly Gln  
 485 490 495  
 Val Ala Lys Gln Gln Val Ala Ser Leu Glu Thr Asn Asp Pro Ile Leu  
 500 505 510 515  
 Gly Phe Gln Ala Thr Asn Glu Arg Leu Phe Val Leu Thr Thr Lys Asn  
 520 525 530  
 Leu Phe Leu Ile Lys Val Asn Thr Glu Asn  
 535 540

<210> 79  
 <211> 99  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -48..-1

<400> 79  
 Met Asp Asn Val Gln Pro Lys Ile Lys His Arg Pro Phe Cys Phe Ser  
 -45 -40 -35  
 Val Lys Gly His Val Lys Met Leu Arg Leu Asp Ile Ile Asn Ser Leu  
 -30 -25 -20  
 Val Thr Thr Val Phe Met Leu Ile Val Ser Val Leu Ala Leu Ile Pro  
 -15 -10 -5  
 Glu Thr Thr Thr Leu Thr Val Gly Gly Gly Val Phe Ala Leu Val Thr  
 1 5 10 15  
 Ala Val Cys Cys Leu Ala Asp Gly Ala Leu Ile Tyr Arg Lys Leu Leu  
 20 25 30  
 Phe Asn Pro Ser Gly Pro Tyr Gln Lys Lys Pro Val His Glu Lys Lys  
 35 40 45  
 Glu Val Leu  
 50

<210> 80  
 <211> 90  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -32..-1

<400> 80  
 Met Pro Cys Leu Asp Gln Gln Leu Thr Val His Ala Leu Pro Cys Pro  
 -30 -25 -20  
 Ala Gln Pro Ser Ser Leu Ala Phe Cys Gln Val Gly Phe Leu Thr Ala  
 -15 -10 -5  
 Gln Pro Ser Pro Pro Arg Arg Arg Asn Gly Lys Asp Arg Tyr Thr Leu  
 1 5 10 15  
 Val Leu Gln His Gln Glu Cys Gln Asp Asp Leu Ala Thr Ser Ser Leu  
 20 25 30  
 Val Tyr Leu Ser Leu Pro Cys Phe Lys Asp Leu Gly Arg Ser Lys His  
 35 40 45

90

Gln Ser Ile Thr Val Ala Asp Thr Asn Lys  
50 55

<210> 81  
<211> 115  
<212> PRT  
<213> Homo Sapiens

<220>  
<221> SIGNAL  
<222> -46...-1

<400> 81  
Met Lys Thr Leu Phe Asn Pro Ala Pro Ala Ile Ala Asp Leu Asp Pro  
-45 -40 -35  
Gln Phe Tyr Thr Leu Ser Asp Val Phe Cys Cys Asn Glu Ser Glu Ala  
-30 -25 -20 -15  
Glu Ile Leu Thr Gly Leu Thr Val Gly Ser Ala Ala Asp Ala Gly Glu  
-10 -5 1  
Ala Ala Leu Val Leu Leu Lys Arg Gly Cys Gln Val Val Ile Ile Thr  
5 10 15  
Leu Gly Ala Glu Gly Cys Val Val Leu Ser Gln Thr Glu Pro Glu Pro  
20 25 30  
Lys His Ile Pro Thr Glu Lys Val Lys Ala Val Asp Thr Thr Cys Arg  
35 40 45 50  
Pro Gly Ser Arg Pro Lys Ser Glu Ala Ala Ser Val Lys Lys Gln Lys  
55 60 65  
His Tyr Lys

<210> 82  
<211> 66  
<212> PRT  
<213> Homo Sapiens

<220>  
<221> SIGNAL  
<222> -19...-1

<400> 82  
Met Lys Pro Leu Leu Val Val Phe Val Phe Leu Phe Leu Trp Asp Pro  
-15 -10 -5  
Val Leu Ala Gly Ile Asn Ser Leu Ser Ser Glu Met His Lys Lys Cys  
1 5 10  
Tyr Lys Asn Gly Ile Cys Arg Leu Glu Cys Tyr Glu Ser Glu Met Leu  
15 20 25  
Val Ala Tyr Cys Met Phe Gln Leu Glu Cys Cys Val Lys Gly Asn Pro  
30 35 40 45  
Ala Pro

<210> 83  
<211> 133  
<212> PRT  
<213> Homo Sapiens

<220>  
<221> SIGNAL  
<222> -21...-1

<400> 83  
Met Ser Cys Ser Leu Lys Phe Thr Leu Ile Val Ile Phe Phe Tyr Cys  
-20 -15 -10  
Trp Leu Ser Ser Ser His Glu Glu Leu Glu Gly Gly Thr Ser Lys Ser



91

```

-5          1          5          10
Phe Asp Leu His Thr Val Ile Met Leu Val Ile Ala Gly Gly Ile Leu
          15          20          25
Ala Ala Leu Leu Leu Leu Ile Val Val Val Leu Cys Leu Tyr Phe Lys
          30          35          40
Ile His Asn Ala Leu Lys Ala Ala Lys Glu Pro Glu Ala Val Ala Val
          45          50          55
Lys Asn His Asn Pro Asp Lys Val Trp Trp Ala Lys Asn Ser Gln Ala
60          65          70          75
Lys Thr Ile Ala Thr Glu Ser Cys Pro Ala Leu Gln Cys Cys Glu Gly
          80          85          90
Tyr Arg Met Cys Ala Ser Phe Asp Ser Leu Pro Pro Cys Cys Cys Asp
          95          100          105
Ile Asn Glu Gly Leu
          110

```

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<210> 84
<211> 140
<212> PRT
<213> Homo Sapiens

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<220>
<221> SIGNAL
<222> -70...-1

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<400> 84
Met Val Leu Thr Lys Pro Leu Gln Arg Asn Gly Ser Met Met Ser Phe
-70          -65          -60          -55
Glu Asn Val Lys Glu Lys Ser Arg Glu Gly Gly Pro His Ala His Thr
          -50          -45          -40
Pro Glu Glu Glu Leu Cys Phe Val Val Thr His Tyr Pro Gln Val Gln
          -35          -30          -25
Thr Thr Leu Asn Leu Phe Phe His Ile Phe Lys Val Leu Thr Gln Pro
          -20          -15          -10
Leu Ser Leu Leu Trp Gly Cys Asp Gln Lys Pro Arg Thr Val Pro Thr
          -5          1          5          10
Leu Gly Asn Gly Ala Trp Asp Thr Cys Gln Gln His Ile Arg Thr Ser
          15          20          25
Ser Trp Thr Ala Asn Thr Leu Val Ile Gln Asn Gln His Ser Arg Glu
          30          35          40
Ser Thr Val Ser Val Cys Leu Phe Met Leu Ile Arg Met Gln His Ile
          45          50          55
Leu Lys Thr Asp Thr Leu Gln Gln Phe Arg Ile Cys
60          65          70

```

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<210> 85
<211> 233
<212> PRT
<213> Homo Sapiens

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<220>
<221> SIGNAL
<222> -32...-1

```

```

<400> 85
Met Ala Thr Pro Pro Phe Arg Leu Ile Arg Lys Met Phe Ser Phe Lys
          -30          -25          -20
Val Ser Arg Trp Met Gly Leu Ala Cys Phe Arg Ser Leu Ala Ala Ser
          -15          -10          -5
Ser Pro Ser Ile Arg Gln Lys Lys Leu Met His Lys Leu Gln Glu Glu
1          5          10          15
Lys Ala Phe Arg Glu Glu Met Lys Ile Phe Arg Glu Lys Ile Glu Asp

```

92

20							25							30						
Phe	Arg	Glu	Glu	Met	Trp	Thr	Phe	Arg	Gly	Lys	Ile	His	Ala	Phe	Arg					
35							40							45						
Gly	Gln	Ile	Leu	Gly	Phe	Trp	Glu	Glu	Glu	Arg	Pro	Phe	Trp	Glu	Glu					
50							55							60						
Glu	Lys	Thr	Phe	Trp	Lys	Glu	Glu	Lys	Ser	Phe	Trp	Glu	Met	Glu	Lys					
65							70							75						
Ser	Phe	Arg	Glu	Glu	Glu	Lys	Thr	Phe	Trp	Lys	Lys	Tyr	Arg	Thr	Phe					
85							90							95						
Trp	Lys	Glu	Asp	Lys	Ala	Phe	Trp	Lys	Glu	Asp	Asn	Ala	Leu	Trp	Glu					
100							105							110						
Arg	Asp	Arg	Asn	Leu	Leu	Gln	Glu	Asp	Lys	Ala	Leu	Trp	Glu	Glu	Glu					
115							120							125						
Lys	Ala	Leu	Trp	Val	Glu	Glu	Arg	Ala	Leu	Leu	Glu	Gly	Glu	Lys	Ala					
130							135							140						
Leu	Trp	Glu	Asp	Lys	Thr	Ser	Leu	Trp	Glu	Glu	Glu	Asn	Ala	Leu	Trp					
145							150							155						
Glu	Glu	Glu	Arg	Ala	Phe	Trp	Met	Glu	Asn	Asn	Gly	His	Ile	Ala	Gly					
165							170							175						
Glu	Gln	Met	Leu	Glu	Asp	Gly	Pro	His	Asn	Ala	Asn	Arg	Gly	Gln	Arg					
180							185							190						
Leu	Leu	Ala	Phe	Ser	Arg	Gly	Arg	Ala												
195							200													

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<210> 86
<211> 83
<212> PRT
<213> Homo Sapiens
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<220>
<221> SIGNAL
<222> -29..-1
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[illegible]

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<210> 87
<211> 215
<212> PRT
<213> Homo Sapiens
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```
<220>
<221> SIGNAL
<222> -41...-1
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<400> 87
Met Val Ser Ala Leu Arg Gly Ala Pro Leu Ile Arg Val His Ser Ser
  -40                      -35                      -30
Pro Val Ser Ser Pro Ser Val Ser Gly Pro Arg Arg Leu Val Ser Cys
-25                      -20                      -15                      -10
Leu Ser Ser Gln Ser Ser Ala Leu Ser Gln Ser Gly Gly Gly Ser Thr
      -5                      1                      5

```

93

Ser Ala Ala Gly Ile Glu Ala Arg Ser Arg Ala Leu Arg Arg Arg Trp  
           10                          15                          20  
 Cys Pro Ala Gly Ile Met Leu Leu Ala Leu Val Cys Leu Leu Ser Cys  
       25                          30                          35  
 Leu Leu Pro Ser Ser Glu Ala Lys Leu Tyr Gly Arg Cys Glu Leu Ala  
 40                          45                          50                          55  
 Arg Val Leu His Asp Phe Gly Leu Asp Gly Tyr Arg Gly Tyr Ser Leu  
                           60                          65                          70  
 Ala Asp Trp Val Cys Leu Ala Tyr Phe Thr Ser Gly Phe Asn Ala Ala  
                           75                          80                          85  
 Ala Leu Asp Tyr Glu Ala Asp Gly Ser Thr Asn Asn Gly Ile Phe Gln  
                           90                          95                          100  
 Ile Asn Ser Arg Arg Trp Cys Ser Asn Leu Thr Pro Asn Val Pro Asn  
 105                          110                          115  
 Val Cys Arg Met Tyr Cys Ser Asp Leu Leu Asn Pro Asn Leu Lys Asp  
 120                          125                          130                          135  
 Thr Val Ile Cys Ala Met Lys Ile Thr Gln Glu Pro Gln Gly Leu Gly  
                           140                          145                          150  
 Tyr Trp Glu Ala Trp Arg His His Cys Gln Gly Lys Asp Leu Thr Glu  
                           155                          160                          165  
 Trp Val Asp Gly Cys Asp Phe  
                           170

&lt;210&gt; 88

&lt;211&gt; 417

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -20...-1

&lt;400&gt; 88

Met Met Gly Ser Pro Val Ser His Leu Leu Ala Gly Phe Cys Val Trp  
 -20                          -15                          -10                          -5  
 Val Val Leu Gly Trp Val Gly Gly Ser Val Pro Asn Leu Gly Pro Ala  
                           1                          5                          10  
 Glu Gln Glu Gln Asn His Tyr Leu Ala Gln Leu Phe Gly Leu Tyr Gly  
                           15                          20                          25  
 Glu Asn Gly Thr Leu Thr Ala Gly Gly Leu Ala Arg Leu Leu His Ser  
                           30                          35                          40  
 Leu Gly Leu Gly Arg Val Gln Gly Leu Arg Leu Gly Gln His Gly Pro  
 45                          50                          55                          60  
 Leu Thr Gly Arg Ala Ala Ser Pro Ala Ala Asp Asn Ser Thr His Arg  
                           65                          70                          75  
 Pro Gln Asn Pro Glu Leu Ser Val Asp Val Trp Ala Gly Met Pro Leu  
                           80                          85                          90  
 Gly Pro Ser Gly Trp Gly Asp Leu Glu Glu Ser Lys Ala Pro His Leu  
                           95                          100                          105  
 Pro Arg Gly Pro Ala Pro Ser Gly Leu Asp Leu Leu His Arg Leu Leu  
                           110                          115                          120  
 Leu Leu Asp His Ser Leu Ala Asp His Leu Asn Glu Asp Cys Leu Asn  
 125                          130                          135                          140  
 Gly Ser Gln Leu Leu Val Asn Phe Gly Leu Ser Pro Ala Ala Pro Leu  
                           145                          150                          155  
 Thr Pro Arg Gln Phe Ala Leu Leu Cys Pro Ala Leu Leu Tyr Gln Ile  
                           160                          165                          170  
 Asp Ser Arg Val Cys Ile Gly Ala Pro Ala Pro Ala Pro Pro Gly Asp  
                           175                          180                          185  
 Leu Leu Ser Ala Leu Leu Gln Ser Ala Leu Ala Val Leu Leu Leu Ser  
                           190                          195                          200  
 Leu Pro Ser Pro Leu Ser Leu Leu Leu Leu Arg Leu Leu Gly Pro Arg

94

205                                      210                                      215                                      220  
 Leu Leu Arg Pro Leu Leu Gly Phe Leu Gly Ala Leu Ala Val Gly Thr  
    225                                      230                                      235  
 Leu Cys Gly Asp Ala Leu Leu His Leu Leu Pro His Ala Gln Glu Gly  
    240                                      245                                      250  
 Arg His Ala Gly Pro Gly Gly Leu Pro Glu Lys Asp Leu Gly Pro Gly  
    255                                      260                                      265  
 Leu Ser Val Leu Gly Gly Leu Phe Leu Leu Phe Val Leu Glu Asn Met  
    270                                      275                                      280  
 Leu Gly Leu Leu Arg His Arg Gly Leu Arg Pro Arg Cys Cys Arg Arg  
 285                                      290                                      295                                      300  
 Lys Arg Arg Asn Leu Glu Thr Arg Asn Leu Asp Pro Glu Asn Gly Ser  
    305                                      310                                      315  
 Gly Met Ala Leu Gln Pro Leu Gln Ala Ala Pro Glu Pro Gly Ala Gln  
    320                                      325                                      330  
 Gly Gln Arg Glu Lys Asn Ser Gln His Pro Pro Ala Leu Ala Pro Pro  
    335                                      340                                      345  
 Gly His Gln Gly His Ser His Gly His Gln Gly Gly Thr Asp Ile Thr  
    350                                      355                                      360  
 Trp Met Val Leu Leu Gly Asp Gly Leu His Asn Leu Thr Asp Gly Leu  
 365                                      370                                      375                                      380  
 Ala Ile Gly Ala Ala Phe Ser Asp Gly Phe Ser Ala Ala Ser Val Pro  
    385                                      390                                      395  
 Pro

<210> 89  
 <211> 366  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -23...-1

<400> 89  
 Met Ala Ser Met Ala Ala Val Leu Thr Trp Ala Leu Ala Leu Leu Ser  
    -20                                      -15                                      -10  
 Ala Phe Ser Ala Thr Gln Ala Arg Lys Gly Phe Trp Asp Tyr Phe Ser  
    -5                                      1                                      5  
 Gln Thr Ser Gly Asp Lys Gly Arg Val Glu Gln Ile His Gln Gln Lys  
 10                                      15                                      20                                      25  
 Met Ala Arg Glu Pro Ala Thr Leu Lys Asp Ser Leu Glu Gln Asp Leu  
    30                                      35                                      40  
 Asn Asn Met Asn Lys Phe Leu Glu Lys Leu Arg Pro Leu Ser Gly Ser  
    45                                      50                                      55  
 Glu Ala Pro Arg Leu Pro Gln Asp Pro Val Gly Met Arg Arg Gln Leu  
    60                                      65                                      70  
 Gln Glu Glu Leu Glu Glu Val Lys Ala Arg Leu Gln Pro Tyr Met Ala  
    75                                      80                                      85  
 Glu Ala His Glu Leu Val Gly Trp Asn Leu Glu Gly Leu Arg Gln Gln  
 90                                      95                                      100                                      105  
 Leu Lys Pro Tyr Thr Met Asp Leu Met Glu Gln Val Ala Leu Arg Val  
    110                                      115                                      120  
 Gln Glu Leu Gln Glu Gln Leu Arg Val Val Gly Glu Asp Thr Lys Ala  
    125                                      130                                      135  
 Gln Leu Leu Gly Gly Val Asp Glu Ala Trp Ala Leu Leu Gln Gly Leu  
    140                                      145                                      150  
 Gln Ser Arg Val Val His His Thr Gly Arg Phe Lys Glu Leu Phe His  
    155                                      160                                      165  
 Pro Tyr Ala Glu Ser Leu Val Ser Gly Ile Gly Arg His Val Gln Glu  
 170                                      175                                      180                                      185  
 Leu His Arg Ser Val Ala Pro His Ala Pro Ala Ser Pro Ala Arg Leu

95

				190					195					200					
Ser	Arg	Cys	Val	Gln	Val	Leu	Ser	Arg	Lys	Leu	Thr	Leu	Lys	Ala	Lys				
			205						210					215					
Ala	Leu	His	Ala	Arg	Ile	Gln	Gln	Asn	Leu	Asp	Gln	Leu	Arg	Glu	Glu				
		220					225					230							
Leu	Ser	Arg	Ala	Phe	Ala	Gly	Thr	Gly	Thr	Glu	Glu	Gly	Ala	Gly	Pro				
	235					240					245								
Asp	Pro	Gln	Met	Leu	Ser	Glu	Glu	Val	Arg	Gln	Arg	Leu	Gln	Ala	Phe				
250				255						260				265					
Arg	Gln	Asp	Thr	Tyr	Leu	Gln	Ile	Ala	Ala	Phe	Thr	Arg	Ala	Ile	Asp				
			270						275					280					
Gln	Glu	Thr	Glu	Glu	Val	Gln	Gln	Gln	Leu	Ala	Pro	Pro	Pro	Pro	Gly				
		285					290						295						
His	Ser	Ala	Phe	Ala	Pro	Glu	Phe	Gln	Gln	Thr	Asp	Ser	Gly	Lys	Val				
		300					305					310							
Leu	Ser	Lys	Leu	Gln	Ala	Arg	Leu	Asp	Asp	Leu	Trp	Glu	Asp	Ile	Thr				
	315			320					325										
His	Ser	Leu	His	Asp	Gln	Gly	His	Ser	His	Leu	Gly	Asp	Pro						
330				335						340									

<210> 90  
 <211> 150  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -45..-1

Met	Val	Leu	Met	Trp	Thr	Ser	Gly	Asp	Ala	Phe	Lys	Thr	Ala	Tyr	Phe				
-45				-40						-35					-30				
Leu	Leu	Lys	Gly	Ala	Pro	Leu	Gln	Phe	Ser	Val	Cys	Gly	Leu	Leu	Gln				
			-25					-20						-15					
Val	Leu	Val	Asp	Leu	Ala	Ile	Leu	Gly	Gln	Ala	Tyr	Ala	Phe	Ala	Pro				
			-10					-5					1						
Pro	Pro	Glu	Ala	Gly	Ala	Pro	Arg	Arg	Ala	Pro	His	Trp	His	Gln	Gly				
	5				10						15								
Pro	Leu	Thr	Val	Gly	Arg	Thr	Arg	Met	Trp	Asp	Arg	Gln	Pro	Arg	Ala				
20				25					30					35					
Leu	Val	Gly	Pro	Asp	Leu	Pro	Ala	Gly	Arg	Val	Gly	Ala	Val	Ala	Pro				
			40					45						50					
Ala	Gly	Val	Ala	Glu	Met	Gly	His	Gly	His	Trp	Gly	Leu	His	Gln	Pro				
		55				60						65							
Leu	Trp	Gly	Val	Ser	Gly	Trp	Ala	Val	Gly	Val	Gly	Leu	Gly	Arg	Cys				
	70					75						80							
Leu	Cys	Ser	Ala	Gly	Thr	Ala	Arg	Val	Asp	Leu	Ala	Pro	Arg	Val	Leu				
	85				90						95								
Asp	Val	Phe	Arg	Met	Thr														
100					105														

<210> 91  
 <211> 308  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -68..-1

<400> 91  
 Met Asp Phe Val Ala Gly Ala Ile Gly Gly Val Cys Gly Val Ala Val

96

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      -65      -60      -55
Gly Tyr Pro Leu Asp Thr Val Lys Val Arg Ile Gln Thr Glu Pro Lys
      -50      -45      -40
Tyr Thr Gly Ile Trp His Cys Val Arg Asp Thr Tyr His Arg Glu Arg
      -35      -30      -25
Val Trp Gly Phe Tyr Arg Gly Leu Ser Leu Pro Val Cys Thr Val Ser
      -20      -15      -10      -5
Leu Val Ser Ser Val Ser Phe Gly Thr Tyr Arg His Cys Leu Ala His
      1      5      10
Ile Cys Arg Leu Arg Tyr Gly Asn Pro Asp Ala Lys Pro Thr Lys Ala
      15      20      25
Asp Ile Thr Leu Ser Gly Cys Ala Ser Gly Leu Val Arg Val Phe Leu
      30      35      40
Thr Ser Pro Thr Glu Val Ala Lys Val Arg Leu Gln Thr Gln Thr Gln
      45      50      55      60
Ala Gln Lys Gln Gln Arg Leu Leu Ser Ala Ser Gly Pro Leu Ala Val
      65      70      75
Pro Pro Met Cys Pro Val Pro Pro Ala Cys Pro Glu Pro Lys Tyr Arg
      80      85      90
Gly Pro Leu His Cys Leu Ala Thr Val Ala Arg Glu Glu Gly Leu Cys
      95      100      105
Gly Leu Tyr Lys Gly Ser Ser Ala Leu Val Leu Arg Asp Gly His Ser
      110      115      120
Phe Ala Thr Tyr Phe Leu Ser Tyr Ala Val Leu Cys Glu Trp Leu Ser
      125      130      135      140
Pro Ala Gly His Ser Arg Pro Asp Val Pro Gly Val Leu Val Ala Gly
      145      150      155
Gly Cys Ala Gly Val Leu Ala Trp Ala Val Ala Thr Pro Met Asp Val
      160      165      170
Ile Lys Ser Arg Leu Gln Ala Asp Gly Gln Gly Gln Arg Arg Tyr Arg
      175      180      185
Gly Leu Leu His Cys Met Val Thr Ser Val Arg Glu Glu Gly Pro Arg
      190      195      200
Val Leu Phe Lys Gly Leu Val Leu Asn Cys Cys Arg Ala Phe Pro Val
      205      210      215      220
Asn Met Val Val Phe Val Ala Tyr Glu Ala Val Leu Arg Leu Ala Arg
      225      230      235
Gly Leu Leu Thr
      240

```

<210> 92  
 <211> 114  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -49..-1

```

<400> 92
Met Glu Lys Pro Leu Phe Pro Leu Val Pro Leu His Trp Phe Gly Phe
      -45      -40      -35
Gly Tyr Thr Ala Leu Val Val Ser Gly Gly Ile Val Gly Tyr Val Lys
      -30      -25      -20
Thr Gly Ser Val Pro Ser Leu Ala Ala Gly Leu Leu Phe Gly Ser Leu
      -15      -10      -5
Ala Gly Leu Gly Ala Tyr Gln Leu Tyr Gln Asp Pro Arg Asn Val Trp
      1      5      10      15
Gly Phe Leu Ala Ala Thr Ser Val Thr Phe Val Gly Val Met Gly Met
      20      25      30
Arg Ser Tyr Tyr Tyr Gly Lys Phe Met Pro Val Gly Leu Ile Ala Gly
      35      40      45

```

97

Ala Ser Leu Leu Met Ala Ala Lys Val Gly Val Arg Met Leu Met Thr  
           50                          55                          60  
 Ser Asp  
       65

<210> 93  
 <211> 382  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -15...-1

<400> 93  
 Met Gly Leu Leu Leu Pro Leu Ala Leu Cys Ile Leu Val Leu Cys Cys  
 -15                          -10                          -5                          1  
 Gly Ala Met Ser Pro Pro Gln Leu Ala Leu Asn Pro Ser Ala Leu Leu  
                           5                          10                          15  
 Ser Arg Gly Cys Asn Asp Ser Asp Val Leu Ala Val Ala Gly Phe Ala  
           20                          25                          30  
 Leu Arg Asp Ile Asn Lys Asp Arg Lys Asp Gly Tyr Val Leu Arg Leu  
           35                          40                          45  
 Asn Arg Val Asn Asp Ala Gln Glu Tyr Arg Arg Gly Gly Leu Gly Ser  
 50                          55                          60                          65  
 Leu Phe Tyr Leu Thr Leu Asp Val Leu Glu Thr Asp Cys His Val Leu  
                           70                          75                          80  
 Arg Lys Lys Ala Trp Gln Asp Cys Gly Met Arg Ile Phe Phe Glu Ser  
                           85                          90                          95  
 Val Tyr Gly Gln Cys Lys Ala Ile Phe Tyr Met Asn Asn Pro Ser Arg  
           100                          105                          110  
 Val Leu Tyr Leu Ala Ala Tyr Asn Cys Thr Leu Arg Pro Val Ser Lys  
           115                          120                          125  
 Lys Lys Ile Tyr Met Thr Cys Pro Asp Cys Pro Ser Ser Ile Pro Thr  
 130                          135                          140                          145  
 Asp Ser Ser Asn His Gln Val Leu Glu Ala Ala Thr Glu Ser Leu Ala  
                           150                          155                          160  
 Lys Tyr Asn Asn Glu Asn Thr Ser Lys Gln Tyr Ser Leu Phe Lys Val  
                           165                          170                          175  
 Thr Arg Ala Ser Ser Gln Trp Val Val Gly Pro Ser Tyr Phe Val Glu  
           180                          185                          190  
 Tyr Leu Ile Lys Glu Ser Pro Cys Thr Lys Ser Gln Ala Ser Ser Cys  
           195                          200                          205  
 Ser Leu Gln Ser Ser Asp Ser Val Pro Val Gly Leu Cys Lys Gly Ser  
 210                          215                          220                          225  
 Leu Thr Arg Thr His Trp Glu Lys Phe Val Ser Val Thr Cys Asp Phe  
                           230                          235                          240  
 Phe Glu Ser Gln Ala Pro Ala Thr Gly Ser Glu Asn Ser Ala Val Asn  
                           245                          250                          255  
 Gln Lys Pro Thr Asn Leu Pro Lys Val Glu Glu Ser Gln Gln Lys Asn  
           260                          265                          270  
 Thr Pro Pro Thr Asp Ser Pro Ser Lys Ala Gly Pro Arg Gly Ser Val  
           275                          280                          285  
 Gln Tyr Leu Pro Asp Leu Asp Asp Lys Asn Ser Gln Glu Lys Gly Pro  
 290                          295                          300                          305  
 Gln Glu Ala Phe Pro Val His Leu Asp Leu Thr Thr Asn Pro Gln Gly  
                           310                          315                          320  
 Glu Thr Leu Asp Ile Ser Phe Leu Phe Leu Glu Pro Met Glu Glu Lys  
                           325                          330                          335  
 Leu Val Val Leu Pro Phe Pro Lys Glu Lys Ala Arg Thr Ala Glu Cys  
           340                          345                          350  
 Pro Gly Pro Ala Gln Asn Ala Ser Pro Leu Val Leu Pro Pro

```

355                                360                                365
<210> 94
<211> 212
<212> PRT
<213> Homo Sapiens

<220>
<221> SIGNAL
<222> -197...-1

<220>
<221> UNSURE
<222> -88
<223> Xaa = Ala,Asp,Gly,Val

<220>
<221> UNSURE
<222> -109
<223> Xaa = Asp,Glu

<400> 94
Met Ala Thr Pro Asn Asn Leu Thr Pro Thr Asn Cys Ser Trp Trp Pro
      -195                                -190                                -185
Ile Ser Ala Leu Glu Ser Asp Ala Ala Lys Pro Ala Glu Ala Pro Asp
      -180                                -175                                -170
Ala Pro Glu Ala Ala Ser Pro Ala His Trp Pro Arg Glu Ser Leu Val
      -165                                -160                                -155                                -150
Leu Tyr His Trp Thr Gln Ser Phe Ser Ser Gln Lys Ala Lys Ile Leu
      -145                                -140                                -135
Glu His Asp Asp Val Ser Tyr Leu Lys Lys Ile Leu Gly Glu Leu Ala
      -130                                -125                                -120
Met Val Leu Asp Gln Ile Glu Ala Xaa Leu Glu Lys Arg Lys Leu Glu
      -115                                -110                                -105
Asn Glu Gly Gln Lys Cys Glu Leu Trp Leu Cys Gly Cys Xaa Phe Thr
      -100                                -95                                -90
Leu Ala Asp Val Leu Leu Gly Ala Thr Leu His Arg Leu Lys Phe Leu
      -85                                -80                                -75                                -70
Gly Leu Ser Lys Lys Tyr Trp Glu Asp Gly Ser Arg Pro Asn Leu Gln
      -65                                -60                                -55
Ser Phe Phe Glu Arg Val Gln Arg Arg Phe Ala Phe Arg Lys Val Leu
      -50                                -45                                -40
Gly Asp Ile His Thr Thr Leu Leu Ser Ala Val Ile Pro Asn Ala Phe
      -35                                -30                                -25
Arg Leu Val Lys Arg Lys Pro Pro Ser Phe Phe Gly Ala Ser Phe Leu
      -20                                -15                                -10
Met Gly Ser Leu Gly Gly Met Gly Tyr Phe Ala Tyr Trp Tyr Leu Lys
      -5                                1                                5                                10
Lys Lys Tyr Ile
      15

<210> 95
<211> 287
<212> PRT
<213> Homo Sapiens

<220>
<221> SIGNAL
<222> -26...-1

<400> 95
Met Gly Ile Gln Thr Ser Pro Val Leu Leu Ala Ser Leu Gly Val Gly

```



99

```

      -25              -20              -15
Leu Val Thr Leu Leu Gly Leu Ala Val Gly Ser Tyr Leu Val Arg Arg
-10              -5              1              5
Ser Arg Arg Pro Gln Val Thr Leu Leu Asp Pro Asn Glu Lys Tyr Leu
      10              15              20
Leu Arg Leu Leu Asp Lys Thr Leu Ser Ala Arg Ser Pro Gly Lys His
      25              30              35
Ile Tyr Leu Ser Thr Arg Ile Asp Gly Ser Leu Val Ile Arg Pro Tyr
      40              45              50
Thr Pro Val Thr Ser Asp Glu Asp Gln Gly Tyr Val Asp Leu Val Ile
55              60              65              70
Lys Val Tyr Leu Lys Gly Val His Pro Lys Phe Pro Glu Gly Gly Lys
      75              80              85
Met Ser Gln Tyr Leu Asp Ser Leu Lys Val Gly Asp Val Val Glu Phe
      90              95              100
Arg Gly Pro Ser Gly Leu Leu Thr Tyr Thr Gly Lys Gly His Phe Asn
      105              110              115
Ile Gln Pro Asn Lys Lys Ser Pro Pro Glu Pro Arg Val Ala Lys Lys
      120              125              130
Leu Gly Met Ile Ala Gly Gly Thr Gly Ile Thr Pro Met Leu Gln Leu
135              140              145              150
Ile Arg Ala Ile Leu Lys Val Pro Glu Asp Pro Thr Gln Cys Phe Leu
      155              160              165
Leu Phe Ala Asn Gln Thr Glu Lys Asp Ile Ile Leu Arg Glu Asp Leu
      170              175              180
Glu Glu Leu Gln Ala Arg Tyr Pro Asn Arg Phe Lys Leu Trp Phe Thr
      185              190              195
Leu Asp His Pro Pro Lys Asp Trp Ala Tyr Ser Lys Gly Phe Val Thr
      200              205              210
Ala Asp Met Ile Arg Glu His Leu Pro Ala Pro Gly Asp Asp Val Leu
215              220              225              230
Val Leu Leu Cys Gly Pro Pro Pro Met Val Gln Leu Ala Cys His Pro
      235              240              245
Asn Leu Asp Lys Leu Gly Tyr Ser Gln Lys Met Arg Phe Thr Tyr
      250              255              260

```

<210> 96  
 <211> 312  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -25...-1

```

<400> 96
Met Ser Asp Leu Leu Leu Leu Gly Leu Ile Gly Gly Leu Thr Leu Leu
-25              -20              -15              -10
Leu Leu Leu Thr Leu Leu Ala Phe Ala Gly Tyr Ser Gly Leu Leu Ala
      -5              1              5
Gly Val Glu Val Ser Ala Gly Ser Pro Pro Ile Arg Asn Val Thr Val
      10              15              20
Ala Tyr Lys Phe His Met Gly Leu Tyr Gly Glu Thr Gly Arg Leu Phe
      25              30              35
Thr Glu Ser Cys Ile Ser Pro Lys Leu Arg Ser Ile Ala Val Tyr Tyr
40              45              50              55
Asp Asn Pro His Met Val Pro Pro Asp Lys Cys Arg Cys Ala Val Gly
      60              65              70
Ser Ile Leu Ser Glu Gly Glu Glu Ser Pro Ser Pro Glu Leu Ile Asp
      75              80              85
Leu Tyr Gln Lys Phe Gly Phe Lys Val Phe Ser Phe Pro Ala Pro Ser
      90              95              100

```

100

```

His Val Val Thr Ala Thr Phe Pro Tyr Thr Thr Ile Leu Ser Ile Trp
  105                110                115
Leu Ala Thr Arg Arg Val His Pro Ala Leu Asp Thr Tyr Ile Lys Glu
  120                125                130                135
Arg Lys Leu Cys Ala Tyr Pro Arg Leu Glu Ile Tyr Gln Glu Asp Gln
                140                145                150
Ile His Phe Met Cys Pro Leu Ala Arg Gln Gly Asp Phe Tyr Val Pro
                155                160                165
Glu Met Lys Glu Thr Glu Trp Lys Trp Arg Gly Leu Val Glu Ala Ile
                170                175                180
Asp Thr Gln Val Asp Gly Thr Gly Ala Asp Thr Met Ser Asp Thr Ser
  185                190                195
Ser Val Ser Leu Glu Val Ser Pro Gly Ser Arg Glu Thr Ser Ala Ala
  200                205                210                215
Thr Leu Ser Pro Gly Ala Ser Ser Arg Gly Trp Asp Asp Gly Asp Thr
                220                225                230
Arg Ser Glu His Ser Tyr Ser Glu Ser Gly Ala Ser Gly Ser Ser Phe
                235                240                245
Glu Glu Leu Asp Leu Glu Gly Glu Gly Pro Leu Gly Glu Ser Arg Leu
                250                255                260
Asp Pro Gly Thr Glu Pro Leu Gly Thr Thr Lys Trp Leu Trp Glu Pro
  265                270                275
Thr Ala Pro Glu Lys Gly Lys Glu
  280                285

```

&lt;210&gt; 97

&lt;211&gt; 226

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -29...-1

&lt;400&gt; 97

```

Met Glu Thr Val Val Ile Val Ala Ile Gly Val Leu Ala Thr Ile Phe
                -25                -20                -15
Leu Ala Ser Phe Ala Ala Leu Val Leu Val Cys Arg Gln Arg Tyr Cys
                -10                -5                1
Arg Pro Arg Asp Leu Leu Gln Arg Tyr Asp Ser Lys Pro Ile Val Asp
  5                10                15
Leu Ile Gly Ala Met Glu Thr Gln Ser Glu Pro Ser Glu Leu Glu Leu
  20                25                30                35
Asp Asp Val Val Ile Thr Asn Pro His Ile Glu Ala Ile Leu Glu Asn
                40                45                50
Glu Asp Trp Ile Glu Asp Ala Ser Gly Leu Met Ser His Cys Ile Ala
                55                60                65
Ile Leu Lys Ile Cys His Thr Leu Thr Glu Lys Leu Val Ala Met Thr
                70                75                80
Met Gly Ser Gly Ala Lys Met Lys Thr Ser Ala Ser Val Ser Asp Ile
  85                90                95
Ile Val Val Ala Lys Arg Ile Ser Pro Arg Val Asp Asp Val Val Lys
  100                105                110                115
Ser Met Tyr Pro Pro Leu Asp Pro Lys Leu Leu Asp Ala Arg Thr Thr
                120                125                130
Ala Leu Leu Leu Ser Val Ser His Leu Val Leu Val Thr Arg Asn Ala
                135                140                145
Cys His Leu Thr Gly Gly Leu Asp Trp Ile Asp Gln Ser Leu Ser Ala
                150                155                160
Ala Glu Glu His Leu Glu Val Leu Arg Glu Ala Ala Leu Ala Ser Glu
  165                170                175
Pro Asp Lys Gly Leu Pro Gly Pro Glu Gly Phe Leu Gln Glu Gln Ser

```

101

180  
Ala Ile

185

190

195

<210> 98  
 <211> 406  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -35..-1

&lt;400&gt; 98

Met Arg Gly Ser Val Glu Cys Thr Trp Gly Trp Gly His Cys Ala Pro  
 -35 -30 -25 -20  
 Ser Pro Leu Leu Leu Trp Thr Leu Leu Leu Phe Ala Ala Pro Phe Gly  
 -15 -10 -5  
 Leu Leu Gly Glu Lys Thr Arg Gln Val Ser Leu Glu Val Ile Pro Asn  
 1 5 10  
 Trp Leu Gly Pro Leu Gln Asn Leu Leu His Ile Arg Ala Val Gly Thr  
 15 20 25  
 Asn Ser Thr Leu His Tyr Val Trp Ser Ser Leu Gly Pro Leu Ala Val  
 30 35 40 45  
 Val Met Val Ala Thr Asn Thr Pro His Ser Thr Leu Ser Val Asn Trp  
 50 55 60  
 Ser Leu Leu Leu Ser Pro Glu Pro Asp Gly Gly Leu Met Val Leu Pro  
 65 70 75  
 Lys Asp Ser Ile Gln Phe Ser Ser Ala Leu Val Phe Thr Arg Leu Leu  
 80 85 90  
 Glu Phe Asp Ser Thr Asn Val Ser Asp Thr Ala Ala Lys Pro Leu Gly  
 95 100 105  
 Arg Pro Tyr Pro Pro Tyr Ser Leu Ala Asp Phe Ser Trp Asn Asn Ile  
 110 115 120 125  
 Thr Asp Ser Leu Asp Pro Ala Thr Leu Ser Ala Thr Phe Gln Gly His  
 130 135 140  
 Pro Met Asn Asp Pro Thr Arg Thr Phe Ala Asn Gly Ser Leu Ala Phe  
 145 150 155  
 Arg Val Gln Ala Phe Ser Arg Ser Ser Arg Pro Ala Gln Pro Pro Arg  
 160 165 170  
 Leu Leu His Thr Ala Asp Thr Cys Gln Leu Glu Val Ala Leu Ile Gly  
 175 180 185  
 Ala Ser Pro Arg Gly Asn Arg Ser Leu Phe Gly Leu Glu Val Ala Thr  
 190 195 200 205  
 Leu Gly Gln Gly Pro Asp Cys Pro Ser Met Gln Glu Gln His Ser Ile  
 210 215 220  
 Asp Asp Glu Tyr Ala Pro Ala Val Phe Gln Leu Asp Gln Leu Leu Trp  
 225 230 235  
 Gly Ser Leu Pro Ser Gly Phe Ala Gln Trp Arg Pro Val Ala Tyr Ser  
 240 245 250  
 Gln Lys Pro Gly Gly Arg Glu Ser Ala Leu Pro Cys Gln Ala Ser Pro  
 255 260 265  
 Leu His Pro Ala Leu Ala Tyr Ser Leu Pro Gln Ser Pro Ile Val Arg  
 270 275 280 285  
 Ala Phe Phe Gly Ser Gln Asn Asn Phe Cys Ala Phe Asn Leu Thr Phe  
 290 295 300  
 Gly Ala Ser Thr Gly Pro Gly Tyr Trp Asp Gln His Tyr Leu Ser Trp  
 305 310 315  
 Ser Met Leu Leu Gly Val Gly Phe Pro Pro Val Asp Gly Leu Ser Pro  
 320 325 330  
 Leu Val Leu Gly Ile Met Ala Val Ala Leu Gly Ala Pro Gly Leu Met  
 335 340 345  
 Leu Leu Gly Gly Gly Leu Val Leu Leu Leu His His Lys Lys Tyr Ser

102

350                                      355  
Glu Tyr Gln Ser Ile Asn  
  370

360

365

```
<210> 99
<211> 120
<212> PRT
<213> Homo Sapiens
```

```
<220>
<221> SIGNAL
<222> -57..-1
```

```

<400> 99
Met Met  Pro Ser Arg Thr Asn Leu Ala Thr Gly Ile Pro Ser Ser Lys
          -55                      -50                      -45
Val Lys  Tyr Ser Arg Leu Ser Ser Thr Asp Asp Gly Tyr Ile Asp Leu
          -40                      -35                      -30
Gln Phe Lys Lys Thr  Pro Pro Lys Ile  Pro Tyr Lys Ala  Ile Ala Leu
          -25                      -20                      -15                      -10
Ala Thr Val Leu Phe Leu Ile Gly Ala Phe Leu Ile Ile Ile Gly Ser
          -5                      1                      5
Leu Leu Leu Ser Gly Tyr Ile Ser Lys Gly Gly Ala Asp Arg Ala Val
          10                      15                      20
Pro Val Leu Ile Ile Gly Ile Leu Val Phe Leu Pro Gly Phe Tyr His
          25                      30                      35
Leu Arg Ile Ala Tyr Tyr Ala Ser Lys Gly Tyr Arg Gly Tyr Ser Tyr
40                      45                      50                      55
Asp Asp Ile Pro Asp Phe Asp Asp
          60

```

```
<210> 100
<211> 210
<212> PRT
<213> Homo Sapiens
```

```
<220>
<221> SIGNAL
<222> -36..-1
```

<400>	100														
Met	Ala	Leu	Pro	Gln	Met	Cys	Asp	Gly	Ser	His	Leu	Ala	Ser	Thr	Leu
-35						-30					-25				
Arg	Tyr	Cys	Met	Thr	Val	Ser	Gly	Thr	Val	Val	Leu	Val	Ala	Gly	Thr
-20					-15					-10					-5
Leu	Cys	Phe	Ala	Trp	Ser	Glu	Gly	Asp	Ala	Thr	Ala	Gln	Pro	Gly	
				1			5					10			
Gln	Leu	Ala	Pro	Pro	Thr	Glu	Tyr	Pro	Val	Pro	Glu	Gly	Pro	Ser	Pro
		15					20					25			
Leu	Leu	Arg	Ser	Val	Ser	Phe	Val	Cys	Cys	Gly	Ala	Gly	Gly	Leu	Leu
30						35					40				
Leu	Leu	Ile	Gly	Leu	Leu	Trp	Ser	Val	Lys	Ala	Ser	Ile	Pro	Gly	Pro
45					50					55					60
Pro	Arg	Trp	Asp	Pro	Tyr	His	Leu	Ser	Arg	Asp	Leu	Tyr	Tyr	Leu	Thr
				65					70					75	
Val	Glu	Ser	Ser	Glu	Lys	Glu	Ser	Cys	Arg	Thr	Pro	Lys	Val	Val	Asp
			80					85					90		
Ile	Pro	Thr	Tyr	Glu	Glu	Ala	Val	Ser	Phe	Pro	Val	Ala	Glu	Gly	Pro
		95					100					105			
Pro	Thr	Pro	Pro	Ala	Tyr	Pro	Thr	Glu	Glu	Ala	Leu	Glu	Pro	Ser	Gly
	110					115					120				
Ser	Arg	Asp	Ala	Leu	Leu	Ser	Thr	Gln	Pro	Ala	Trp	Pro	Pro	Pro	Ser

103

125 130 135 140  
 Tyr Glu Ser Ile Ser Leu Ala Leu Asp Ala Val Ser Ala Glu Thr Thr  
 145 150 155  
 Pro Ser Ala Thr Arg Ser Cys Ser Gly Leu Val Gln Thr Ala Arg Gly  
 160 165 170  
 Gly Ser

<210> 101  
 <211> 251  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -243...-1

<400> 101  
 Met Ala His Arg Leu Gln Ile Arg Leu Leu Thr Trp Asp Val Lys Asp  
 -240 -235 -230  
 Thr Leu Leu Arg Leu Arg His Pro Leu Gly Glu Ala Tyr Ala Thr Lys  
 -225 -220 -215  
 Ala Arg Ala His Gly Leu Glu Val Glu Pro Ser Ala Leu Glu Gln Gly  
 -210 -205 -200  
 Phe Arg Gln Ala Tyr Arg Ala Gln Ser His Ser Phe Pro Asn Tyr Gly  
 -195 -190 -185 -180  
 Leu Ser His Gly Leu Thr Ser Arg Gln Trp Trp Leu Asp Val Val Leu  
 -175 -170 -165  
 Gln Thr Phe His Leu Ala Gly Val Gln Asp Ala Gln Ala Val Ala Pro  
 -160 -155 -150  
 Ile Ala Glu Gln Leu Tyr Lys Asp Phe Ser His Pro Cys Thr Trp Gln  
 -145 -140 -135  
 Val Leu Asp Gly Ala Glu Asp Thr Leu Arg Glu Cys Arg Thr Arg Gly  
 -130 -125 -120  
 Leu Arg Leu Ala Val Ile Ser Asn Phe Asp Arg Arg Leu Glu Gly Ile  
 -115 -110 -105 -100  
 Leu Glu Gly Leu Gly Leu Arg Glu His Phe Asp Phe Val Leu Thr Ser  
 -95 -90 -85  
 Glu Ala Ala Gly Trp Pro Lys Pro Asp Pro Arg Ile Phe Gln Glu Ala  
 -80 -75 -70  
 Leu Arg Leu Ala His Met Glu Pro Val Val Ala Ala His Val Gly Asp  
 -65 -60 -55  
 Asn Tyr Leu Cys Asp Tyr Gln Gly Pro Arg Ala Val Gly Met His Ser  
 -50 -45 -40  
 Phe Leu Val Val Gly Pro Gln Ala Leu Asp Pro Val Val Arg Asp Ser  
 -35 -30 -25 -20  
 Val Pro Lys Glu His Ile Leu Pro Ser Leu Ala His Leu Leu Pro Ala  
 -15 -10 -5  
 Leu Asp Cys Leu Glu Gly Ser Thr Pro Gly Leu  
 1 5

<210> 102  
 <211> 126  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -24...-1

<400> 102  
 Met Asp Lys Ser Leu Leu Leu Glu Leu Pro Ile Leu Leu Cys Cys Phe  
 -20 -15 -10

104

```

Arg Ala Leu Ser Gly Ser Leu Ser Met Arg Asn Asp Ala Val Asn Glu
      -5              1              5
Ile Val Ala Val Lys Asn Asn Phe Pro Val Ile Glu Ile Ile Gln Cys
      10              15              20
Arg Met Cys His Leu Gln Phe Pro Gly Glu Lys Cys Ser Arg Gly Arg
      25              30              35              40
Gly Ile Cys Thr Ala Thr Thr Glu Glu Ala Cys Met Val Gly Arg Met
      45              50              55
Phe Lys Arg Asp Gly Asn Pro Trp Leu Thr Phe Met Gly Cys Leu Lys
      60              65              70
Asn Cys Ala Asp Val Lys Gly Ile Arg Trp Ser Val Tyr Leu Val Asn
      75              80              85
Phe Arg Cys Cys Arg Ser His Asp Leu Cys Asn Glu Asp Leu
      90              95              100

```

<210> 103  
 <211> 133  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -44...-1

```

<400> 103
Met Asp Arg Arg Ala Thr Ser Phe Pro Pro Leu Pro Ala Lys Glu Arg
      -40              -35              -30
Arg Ala Gly Ile Ser Ser Ala Leu Pro Cys Pro Pro Thr Met Ser Leu
      -25              -20              -15
Ser Asp Ser Leu Trp Ser Pro His Cys Ser Trp Ser Glu Arg Pro His
      -10              -5              1
Ser Phe Ser His Trp Arg Gln Pro Arg Met Gly Ser Ser Gly Gly Ser
      5              10              15              20
Leu Asp Tyr Val Ser Phe Lys His Trp Ile His Ser Ser Arg Ser Lys
      25              30              35
Gly Lys Ile Ala Ala Leu Glu Ala Gly Leu Phe Ile Ser Cys Leu Gly
      40              45              50
Asp Ala Pro Arg Gly Leu Asn Ala Ser Gln Gly Asn Gln Arg Lys Asn
      55              60              65
Met Val Cys Phe Arg Gly Gly Val Ala Ser Leu Ala Leu Pro Ser Leu
      70              75              80
Thr Pro Ser Cys Leu
      85

```

<210> 104  
 <211> 221  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -28...-1

```

<400> 104
Met Glu Ala Gly Gly Phe Leu Asp Ser Leu Ile Tyr Gly Ala Cys Val
      -25              -20              -15
Val Phe Thr Leu Gly Met Phe Ser Ala Gly Leu Ser Asp Leu Arg His
      -10              -5              1
Met Arg Met Thr Arg Ser Val Asp Asn Val Gln Phe Leu Pro Phe Leu
      5              10              15              20
Thr Thr Glu Val Asn Asn Leu Gly Trp Leu Ser Tyr Gly Ala Leu Lys
      25              30              35

```

105

Gly Asp Gly Ile Leu Ile Val Val Asn Thr Val Gly Ala Ala Leu Gln  
                   40                  45                  50  
 Thr Leu Tyr Ile Leu Ala Tyr Leu His Tyr Cys Pro Arg Lys Arg Val  
                   55                  60                  65  
 Val Leu Leu Gln Thr Ala Thr Leu Leu Gly Val Leu Leu Leu Gly Tyr  
                   70                  75                  80  
 Gly Tyr Phe Trp Leu Leu Val Pro Asn Pro Glu Ala Arg Leu Gln Gln  
 85                  90                  95                  100  
 Leu Gly Leu Phe Cys Ser Val Phe Thr Ile Ser Met Tyr Leu Ser Pro  
                   105                  110                  115  
 Leu Ala Asp Leu Ala Lys Val Ile Gln Thr Lys Ser Thr Gln Cys Leu  
                   120                  125                  130  
 Ser Tyr Pro Leu Thr Ile Ala Thr Leu Leu Thr Ser Ala Ser Trp Cys  
                   135                  140                  145  
 Leu Tyr Gly Phe Arg Leu Arg Asp Pro Tyr Ile Met Val Ser Asn Phe  
                   150                  155                  160  
 Pro Gly Ile Val Thr Ser Phe Ile Arg Phe Trp Leu Phe Trp Lys Tyr  
 165                  170                  175                  180  
 Pro Gln Glu Gln Asp Arg Asn Tyr Trp Leu Leu Gln Thr  
                   185                  190

&lt;210&gt; 105

&lt;211&gt; 352

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -23...-1

&lt;400&gt; 105

Met Glu Ser Gly Gly Arg Pro Ser Leu Cys Gln Phe Ile Leu Leu Gly  
                   -20                  -15                  -10  
 Thr Thr Ser Val Val Thr Ala Ala Leu Tyr Ser Val Tyr Arg Gln Lys  
                   -5                  1                  5  
 Ala Arg Val Ser Gln Glu Leu Lys Gly Ala Lys Lys Val His Leu Gly  
 10                  15                  20                  25  
 Glu Asp Leu Lys Ser Ile Leu Ser Glu Ala Pro Gly Lys Cys Val Pro  
                   30                  35                  40  
 Tyr Ala Val Ile Glu Gly Ala Val Arg Ser Val Lys Glu Thr Leu Asn  
                   45                  50                  55  
 Ser Gln Phe Val Glu Asn Cys Lys Gly Val Ile Gln Arg Leu Thr Leu  
                   60                  65                  70  
 Gln Glu His Lys Met Val Trp Asn Arg Thr Thr His Leu Trp Asn Asp  
                   75                  80                  85  
 Cys Ser Lys Ile Ile His Gln Arg Thr Asn Thr Val Pro Phe Asp Leu  
 90                  95                  100                  105  
 Val Pro His Glu Asp Gly Val Asp Val Ala Val Arg Val Leu Lys Pro  
                   110                  115                  120  
 Leu Asp Ser Val Asp Leu Gly Leu Glu Thr Val Tyr Glu Lys Phe His  
                   125                  130                  135  
 Pro Ser Ile Gln Ser Phe Thr Asp Val Ile Gly His Tyr Ile Ser Gly  
                   140                  145                  150  
 Glu Arg Pro Lys Gly Ile Gln Glu Thr Glu Glu Met Leu Lys Val Gly  
                   155                  160                  165  
 Ala Thr Leu Thr Gly Val Gly Glu Leu Val Leu Asp Asn Asn Ser Val  
 170                  175                  180                  185  
 Arg Leu Gln Pro Pro Lys Gln Gly Met Gln Tyr Tyr Leu Ser Ser Gln  
                   190                  195                  200  
 Asp Phe Asp Ser Leu Leu Gln Arg Gln Glu Ser Ser Val Arg Leu Trp  
                   205                  210                  215  
 Lys Val Leu Ala Leu Val Phe Gly Phe Ala Thr Cys Ala Thr Leu Phe

106

	220		225		230										
Phe	Ile	Leu	Arg	Lys	Gln	Tyr	Leu	Gln	Arg	Gln	Glu	Arg	Leu	Arg	Leu
	235					240					245				
Lys	Gln	Met	Gln	Glu	Glu	Phe	Gln	Glu	His	Glu	Ala	Gln	Leu	Leu	Ser
250					255					260				265	
Arg	Ala	Lys	Pro	Glu	Asp	Arg	Glu	Ser	Leu	Lys	Ser	Ala	Cys	Val	Val
			270					275					280		
Cys	Leu	Ser	Ser	Phe	Lys	Ser	Cys	Val	Phe	Leu	Glu	Cys	Gly	His	Val
			285				290					295			
Cys	Ser	Cys	Thr	Glu	Cys	Tyr	Arg	Ala	Leu	Pro	Glu	Pro	Lys	Lys	Cys
	300					305				310					
Pro	Ile	Cys	Arg	Gln	Ala	Ile	Thr	Arg	Val	Ile	Pro	Leu	Tyr	Asn	Ser
315						320					325				

&lt;210&gt; 106

&lt;211&gt; 385

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -184...-1

&lt;400&gt; 106

Met	Trp	Thr	Phe	Ser	Tyr	Ile	Gly	Phe	Pro	Val	Glu	Leu	Asn	Thr	Val
			-180					-175					-170		
Tyr	Phe	Ile	Gly	Ala	His	Lys	Ile	Pro	Asn	Ala	Asn	Met	Asn	Glu	Asp
			-165				-160					-155			
Gly	Pro	Ser	Met	Ser	Val	Asn	Phe	Thr	Ser	Pro	Gly	Cys	Leu	Asp	His
			-150				-145				-140				
Ile	Met	Lys	Tyr	Lys	Lys	Lys	Cys	Val	Lys	Ala	Gly	Ser	Leu	Trp	Asp
	-135				-130						-125				
Pro	Asn	Ile	Thr	Ala	Cys	Lys	Lys	Asn	Glu	Glu	Thr	Val	Glu	Val	Asn
-120				-115					-110				-105		
Phe	Thr	Thr	Thr	Pro	Leu	Gly	Asn	Arg	Tyr	Met	Ala	Leu	Ile	Gln	His
			-100				-95						-90		
Ser	Thr	Ile	Ile	Gly	Phe	Ser	Gln	Val	Phe	Glu	Pro	His	Gln	Lys	Lys
			-85			-80						-75			
Gln	Thr	Arg	Ala	Ser	Val	Val	Ile	Pro	Val	Thr	Gly	Asp	Ser	Glu	Gly
		-70				-65					-60				
Ala	Thr	Val	Gln	Leu	Thr	Pro	Tyr	Phe	Pro	Thr	Cys	Gly	Ser	Asp	Cys
	-55				-50				-45						
Ile	Arg	His	Lys	Gly	Thr	Val	Val	Leu	Cys	Pro	Gln	Thr	Gly	Val	Pro
-40				-35				-30					-25		
Phe	Pro	Leu	Asp	Asn	Asn	Lys	Ser	Lys	Pro	Gly	Gly	Trp	Leu	Pro	Leu
			-20				-15					-10			
Leu	Leu	Leu	Ser	Leu	Leu	Val	Ala	Thr	Trp	Val	Leu	Val	Ala	Gly	Ile
		-5					1				5				
Tyr	Leu	Met	Trp	Arg	His	Glu	Arg	Ile	Lys	Lys	Thr	Ser	Phe	Ser	Thr
10				15						20					
Thr	Thr	Leu	Leu	Pro	Pro	Ile	Lys	Val	Leu	Val	Val	Tyr	Pro	Ser	Glu
25				30					35				40		
Ile	Cys	Phe	His	His	Thr	Ile	Cys	Tyr	Phe	Thr	Glu	Phe	Leu	Gln	Asn
			45				50						55		
His	Cys	Arg	Ser	Glu	Val	Ile	Leu	Glu	Lys	Trp	Gln	Lys	Lys	Lys	Ile
		60					65					70			
Ala	Glu	Met	Gly	Pro	Val	Gln	Trp	Leu	Ala	Thr	Gln	Lys	Lys	Ala	Ala
	75					80					85				
Asp	Lys	Val	Val	Phe	Leu	Leu	Ser	Asn	Asp	Val	Asn	Ser	Val	Cys	Asp
	90				95					100					
Gly	Thr	Cys	Gly	Lys	Ser	Glu	Gly	Ser	Pro	Ser	Glu	Asn	Ser	Gln	Asp
105				110						115				120	



107

Leu Phe Pro Leu Ala Phe Asn Leu Phe Cys Ser Asp Leu Arg Ser Gln  
                           125                          130                          135  
 Ile His Leu His Lys Tyr Val Val Val Tyr Phe Arg Glu Ile Asp Thr  
                           140                          145                          150  
 Lys Asp Asp Tyr Asn Ala Leu Ser Val Cys Pro Lys Tyr His Leu Met  
                           155                          160                          165  
 Lys Asp Ala Thr Ala Phe Cys Ala Glu Leu Leu His Val Lys Gln Gln  
                           170                          175                          180  
 Val Ser Ala Gly Lys Arg Ser Gln Ala Cys His Asp Gly Cys Cys Ser  
 185                          190                          195                          200  
 Leu

<210> 107  
 <211> 69  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -23...-1

<400> 107  
 Met Asn Leu Met Trp Thr Leu Leu Leu Phe Leu Leu Leu Asp Val Thr  
                           -20                          -15                          -10  
 Val Phe Ile Pro Ala Leu Pro Phe Ser Thr Arg His Ile Asp Asn Pro  
                           -5                          1                          5  
 Arg Ser Trp Val Pro Arg Gly His His Arg Tyr Cys Asp Val Met Met  
 10                          15                          20                          25  
 Arg Arg Arg Trp Leu Ile Tyr Arg Gly Lys Cys Glu Gln Ile His Thr  
                           30                          35                          40  
 Phe Ile His Arg Ile  
                           45

<210> 108  
 <211> 108  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -49...-1

<400> 108  
 Met Asn Lys Thr His Lys Asp Cys Ser Ser Pro Gln Tyr Ser Ile Tyr  
                           -45                          -40                          -35  
 Asn Ile Leu Asn Glu Leu Pro Thr Arg Pro Ile Ile Leu Ser Cys Ser  
                           -30                          -25                          -20  
 Gln Ile Ser Cys Leu Leu Leu Val Ser Thr Trp Ser Ala Asp Leu Met  
                           -15                          -10                          -5  
 Ser Tyr Arg Pro Val Thr Lys Pro Ser Gln Arg Cys Thr Ser Pro Ala  
                           1                          5                          10                          15  
 Gln Ser Met Thr Val Asn Leu Thr Lys Asp Val Gly Phe Tyr Glu Asp  
                           20                          25                          30  
 Thr Gln Ser Ile Arg Ile Thr Leu Ser Glu Ile Ser Gln Ala Gln Lys  
                           35                          40                          45  
 Asp Thr Tyr Phe Ile Ile Ser Cys Ile Cys Gly Ile  
                           50                          55

<210> 109  
 <211> 108  
 <212> PRT  
 <213> Homo Sapiens

108

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -28...-1

&lt;400&gt; 109

```

Met Tyr Phe His Phe Leu Gly Ala Gly Ala Ile Leu Ile Pro Arg Leu
      -25              -20              -15
Asp Ile Val Ile Ser Phe Val Gly Ala Val Ser Ser Ser Thr Leu Ala
      -10              -5              1
Leu Ile Leu Pro Pro Leu Val Glu Ile Leu Thr Phe Ser Lys Glu His
5              10              15              20
Tyr Asn Ile Trp Met Val Leu Lys Asn Ile Ser Ile Ala Phe Thr Gly
      25              30              35
Val Val Gly Phe Leu Leu Gly Thr Tyr Ile Thr Val Glu Glu Ile Ile
      40              45              50
Tyr Pro Thr Pro Lys Val Val Ala Gly Thr Pro Gln Ser Pro Phe Leu
      55              60              65
Asn Leu Asn Ser Thr Cys Leu Thr Ser Gly Leu Lys
      70              75              80

```

&lt;210&gt; 110

&lt;211&gt; 125

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -37...-1

&lt;400&gt; 110

```

Met Val Cys Glu Asp Ala Pro Ser Phe Gln Met Ala Trp Glu Ser Gln
      -35              -30              -25
Met Ala Trp Glu Arg Gly Pro Ala Leu Leu Cys Cys Val Leu Ser Ala
      -20              -15              -10
Ser Gln Leu Ser Ser Gln Asp Gln Asp Pro Leu Gly His Ile Lys Ser
-5              1              5              10
Leu Leu Tyr Pro Phe Gly Phe Pro Val Glu Leu Pro Arg Pro Gly Pro
      15              20              25
Thr Gly Ala Tyr Lys Lys Val Lys Asn Gln Asn Gln Thr Ser Ser
      30              35              40
Glu Leu Leu Arg Lys Gln Thr Ser His Phe Asn Gln Arg Gly His Arg
      45              50              55
Ala Arg Ser Lys Leu Leu Ala Ser Arg Gln Ile Pro Asp Arg Thr Phe
60              65              70              75
Lys Cys Gly Lys Trp Leu Pro Gln Val Pro Ser Pro Val
      80              85

```

&lt;210&gt; 111

&lt;211&gt; 169

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; SIGNAL

&lt;222&gt; -88...-1

&lt;400&gt; 111

```

Met Lys Gly Gly Ile Ser Asn Val Trp Phe Asp Arg Phe Lys Ile Thr
      -85              -80              -75
Asn Asp Cys Pro Glu His Leu Glu Ser Ile Asp Val Met Cys Gln Val
      -70              -65              -60

```

109

```

Leu Thr Asp Leu Ile Asp Glu Glu Val Lys Ser Gly Ile Lys Lys Asn
-55 -50 -45
Arg Ile Leu Ile Gly Gly Phe Ser Met Gly Gly Cys Met Ala Met His
-40 -35 -30 -25
Leu Ala Tyr Arg Asn His Gln Asp Val Ala Gly Val Phe Ala Leu Ser
-20 -15 -10
Ser Phe Leu Asn Lys Ala Ser Ala Val Tyr Gln Ala Leu Gln Lys Ser
-5 1 5
Asn Gly Val Leu Pro Glu Leu Phe Gln Cys His Gly Thr Ala Asp Glu
10 15 20
Leu Val Leu His Ser Trp Ala Glu Glu Thr Asn Ser Met Leu Lys Ser
25 30 35 40
Leu Gly Val Thr Thr Lys Phe His Ser Phe Pro Asn Val Tyr His Glu
45 50 55
Leu Ser Lys Thr Glu Leu Asp Ile Leu Lys Leu Trp Ile Leu Thr Lys
60 65 70
Leu Pro Gly Glu Met Glu Lys Gln Lys
75 80

```

```

<210> 112
<211> 82
<212> PRT
<213> Homo Sapiens

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<220>
<221> SIGNAL
<222> -56...-1

```

```

<400> 112
Met Lys Ala Val Trp His Phe Cys Leu Ser His Lys Ser Ser Leu Val
-55 -50 -45
Ile Val Leu Lys Thr Ala Gly Trp Ile Pro Gln Ala Gly Thr Leu Ile
-40 -35 -30 -25
Pro Gly Ser Arg Glu Glu Ser Arg Ser Asp Ser Gln Met Ile Met Leu
-20 -15 -10
Val Cys Phe Asn Leu Ser Arg Gly Cys Leu Lys Lys Val Phe Ile Ile
-5 1 5
Ser Val Leu Pro Asp Pro Glu Thr Ile Leu Leu Gly Lys Thr Val Gly
10 15 20
Ile Ala
25

```

```

<210> 113
<211> 251
<212> PRT
<213> Homo Sapiens

```

```

<220>
<221> SIGNAL
<222> -20...-1

```

```

<400> 113
Met Asp Lys Val Gln Ser Gly Phe Leu Ile Leu Phe Leu Phe Leu Met
-20 -15 -10 -5
Glu Cys Gln Leu His Leu Cys Leu Pro Tyr Ala Asp Gly Leu His Pro
1 5 10
Thr Gly Asn Ile Thr Gly Leu Pro Gly Ser Phe Asn His Trp Phe Tyr
15 20 25
Val Thr Gln Gly Glu Leu Lys Ser Cys Phe Arg Gly Asp Lys Lys Lys
30 35 40
Val Ile Thr Phe His Arg Lys Lys Phe Ser Phe Gln Gly Ser Lys Arg
45 50 55 60

```

110

```

Ser Gln Pro Pro Arg Asn Ile Thr Lys Glu Pro Lys Val Phe Phe His
      65      70      75
Lys Thr Gln Leu Pro Gly Ile Gln Gly Ala Ala Ser Arg Ser Thr Ala
      80      85      90
Ala Ser Pro Thr Asn Pro Met Lys Phe Leu Arg Asn Lys Ala Ile Ile
      95     100     105
Arg His Arg Pro Ala Leu Val Lys Val Ile Leu Ile Ser Ser Val Ala
     110     115     120
Phe Ser Ile Ala Leu Ile Cys Gly Met Ala Ile Ser Tyr Met Ile Tyr
    125     130     135     140
Arg Leu Ala Gln Ala Glu Glu Arg Gln Gln Leu Glu Ser Leu Tyr Lys
      145     150     155
Asn Leu Arg Ile Pro Leu Leu Gly Asp Glu Glu Glu Gly Ser Glu Asp
     160     165     170
Glu Gly Glu Ser Thr His Leu Leu Pro Lys Asn Glu Asn Glu Leu Glu
     175     180     185
Lys Phe Ile His Ser Val Ile Ile Ser Lys Arg Ser Lys Asn Ile Lys
     190     195     200
Lys Lys Leu Lys Glu Glu Gln Asn Ser Val Thr Glu Asn Lys Thr Lys
    205     210     215     220
Asn Ala Ser His Asn Gly Lys Met Glu Asp Leu
      225     230

```

```

<210> 114
<211> 305
<212> PRT
<213> Homo Sapiens

```

```

<220>
<221> SIGNAL
<222> -34...-1

```

```

<400> 114
Met Ser Phe Leu Arg Ile Thr Pro Ser Thr His Ser Ser Val Ser Ser
      -30      -25      -20
Gly Leu Leu Arg Leu Ser Ile Phe Leu Leu Leu Ser Phe Pro Asp Ser
     -15     -10     -5
Asn Gly Lys Ala Ile Trp Thr Ala His Leu Asn Ile Thr Phe Gln Val
      1      5      10
Gly Asn Glu Ile Thr Ser Glu Leu Gly Glu Ser Gly Val Phe Gly Asn
    15     20     25     30
His Ser Pro Leu Glu Arg Val Ser Gly Val Val Ala Leu Pro Glu Glu
      35     40     45
Trp Asn Gln Asn Ala Cys His Pro Leu Thr Asn Phe Ser Arg Pro Lys
     50     55     60
Gln Ala Asp Ser Trp Leu Ala Leu Ile Glu Arg Gly Gly Cys Thr Phe
     65     70     75
Thr His Lys Ile Asn Val Ala Ala Glu Lys Gly Ala Asn Gly Val Ile
     80     85     90
Ile Tyr Asn Tyr Gln Gly Thr Gly Ser Lys Val Phe Pro Met Ser His
    95     100     105     110
Gln Gly Thr Glu Asn Ile Val Ala Val Met Ile Ser Asn Leu Lys Gly
      115     120     125
Met Glu Ile Leu His Ser Ile Gln Lys Gly Val Tyr Val Thr Val Ile
     130     135     140
Ile Glu Val Gly Arg Met His Met Gln Trp Val Ser His Tyr Ile Met
     145     150     155
Tyr Leu Phe Thr Phe Leu Ala Ala Thr Ile Ala Tyr Phe Tyr Leu Asp
    160     165     170
Cys Val Trp Arg Leu Thr Pro Arg Val Pro Asn Ser Phe Thr Arg Arg
    175     180     185     190
Arg Ser Gln Ile Lys Thr Asp Val Lys Lys Ala Ile Asp Gln Leu Gln

```

111

				195					200					205					
Leu	Arg	Val	Leu	Lys	Glu	Gly	Asp	Glu	Glu	Leu	Asp	Leu	Asn	Glu	Asp				
			210					215					220						
Asn	Cys	Val	Val	Cys	Phe	Asp	Thr	Tyr	Lys	Pro	Gln	Asp	Val	Val	Arg				
		225					230					235							
Ile	Leu	Thr	Cys	Lys	His	Phe	Phe	His	Lys	Ala	Cys	Ile	Asp	Pro	Trp				
		240				245					250								
Leu	Leu	Ala	His	Arg	Thr	Cys	Pro	Met	Cys	Lys	Cys	Asp	Ile	Leu	Lys				
255					260					265					270				
Thr																			

<210> 115  
 <211> 61  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -42...-1

Met	Thr	Asp	Leu	Asp	Leu	Met	Ile	Asn	Phe	Thr	Phe	Pro	Ile	Gln	Trp				
		-40					-35					-30							
Val	Asn	Gln	Asn	Arg	Met	Ala	Tyr	Tyr	Ser	Leu	Lys	Pro	Leu	Leu	Pro				
		-25				-20					-15								
Cys	Ser	Ser	Val	Leu	Thr	Cys	Gly	Gln	Ala	Ser	Gln	Asp	Leu	Leu	Thr				
-10					-5					1				5					
Ser	Ala	Thr	Ser	Val	Thr	Gly	Met	Glu	Lys	Ile	Glu	Ala							
			10					15											

<210> 116  
 <211> 113  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -15...-1

Met	Asn	Phe	Tyr	Leu	Leu	Leu	Ala	Ser	Ser	Ile	Leu	Cys	Ala	Leu	Ile				
-15				-10						-5				1					
Val	Phe	Trp	Lys	Tyr	Arg	Arg	Phe	Gln	Arg	Asn	Thr	Gly	Glu	Met	Ser				
		5					10					15							
Ser	Asn	Ser	Thr	Ala	Leu	Ala	Leu	Val	Arg	Pro	Ser	Ser	Ser	Gly	Leu				
		20				25					30								
Ile	Asn	Ser	Asn	Thr	Asp	Asn	Asn	Leu	Ala	Val	Tyr	Asp	Leu	Ser	Arg				
	35				40					45									
Asp	Ile	Leu	Asn	Asn	Phe	Pro	His	Ser	Ile	Ala	Arg	Gln	Lys	Arg	Ile				
50				55					60					65					
Leu	Val	Asn	Leu	Ser	Met	Val	Glu	Asn	Lys	Leu	Val	Glu	Leu	Glu	His				
			70					75						80					
Thr	Leu	Leu	Ser	Lys	Gly	Phe	Arg	Gly	Ala	Ser	Pro	His	Arg	Lys	Ser				
			85					90						95					
Thr																			

<210> 117  
 <211> 101  
 <212> PRT  
 <213> Homo Sapiens

<220>

112

<221> SIGNAL  
 <222> -30...-1

<400> 117

Met	Glu	Arg	Pro	Arg	Ser	Pro	Gln	Cys	Ser	Ala	Pro	Ala	Ser	Ala	Ser
-30					-25					-20					-15
Ala	Ser	Val	Thr	Leu	Ala	Gln	Leu	Leu	Gln	Leu	Val	Gln	Gln	Gly	Gln
				-10					-5					1	
Glu	Leu	Pro	Gly	Leu	Glu	Lys	Arg	His	Ile	Ala	Ala	Ile	His	Gly	Glu
		5				10						15			
Pro	Thr	Ala	Ser	Arg	Leu	Pro	Arg	Arg	Pro	Lys	Pro	Trp	Glu	Ala	Ala
	20					25					30				
Ala	Leu	Ala	Glu	Ser	Leu	Pro	Pro	Pro	Thr	Leu	Arg	Ile	Gly	Thr	Ala
35					40					45					50
Pro	Ala	Glu	Pro	Gly	Leu	Val	Glu	Ala	Ala	Thr	Ala	Pro	Ser	Ser	Trp
				55					60					65	
His	Thr	Val	Gly	Pro											
				70											

<210> 118  
 <211> 97  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -90...-1

<220>  
 <221> UNSURE  
 <222> -39  
 <223> Xaa = His,Gln

<400> 118

Met	Asn	Gln	Glu	Asn	Pro	Pro	Pro	Tyr	Pro	Gly	Pro	Gly	Pro	Thr	Ala
-90					-85					-80					-75
Pro	Tyr	Pro	Pro	Tyr	Pro	Pro	Gln	Pro	Met	Gly	Pro	Gly	Pro	Met	Gly
				-70					-65					-60	
Gly	Pro	Tyr	Pro	Pro	Pro	Gln	Gly	Tyr	Pro	Tyr	Gln	Gly	Tyr	Leu	Gln
			-55				-50						-45		
Tyr	Gly	Trp	Xaa	Gly	Gly	Pro	Gln	Glu	Pro	Pro	Lys	Thr	Thr	Val	Tyr
		-40				-35					-30				
Val	Val	Glu	Asp	Gln	Arg	Arg	Asp	Glu	Leu	Gly	Pro	Ser	Thr	Cys	Leu
	-25				-20					-15					
Thr	Ala	Cys	Trp	Thr	Ala	Leu	Cys	Cys	Cys	Cys	Leu	Trp	Asp	Met	Leu
-10					-5					1				5	
Thr															

<210> 119  
 <211> 101  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -25...-1

<400> 119

Met	Val	Asp	Arg	Glu	Leu	Ala	Asp	Ile	His	Glu	Asp	Ala	Lys	Thr	Cys
-25					-20					-15					-10
Leu	Val	Leu	Cys	Ser	Arg	Val	Leu	Ser	Val	Ile	Ser	Val	Lys	Glu	Ile
			-5					1					5		

113

Lys Thr Gln Leu Ser Leu Gly Arg His Pro Ile Ile Ser Asn Trp Phe  
           10                  15                  20  
 Asp Tyr Ile Pro Ser Thr Arg Tyr Lys Asp Pro Cys Glu Leu Leu His  
           25                  30                  35  
 Leu Cys Arg Leu Thr Ile Arg Asn Gln Leu Leu Thr Asn Asn Met Leu  
 40                  45                  50                  55  
 Pro Asp Gly Ile Phe Ser Leu Leu Ile Pro Ala Arg Leu Gln Asn Tyr  
                   60                  65                  70  
 Leu Asn Leu Glu Ile  
                   75

<210> 120  
 <211> 152  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -101...-1

<400> 120  
 Met Asp Asn Val Gln Pro Lys Ile Lys His Arg Pro Phe Cys Phe Ser  
           -100                  -95                  -90  
 Val Lys Gly His Val Lys Met Leu Arg Leu Ala Leu Thr Val Thr Ser  
           -85                  -80                  -75                  -70  
 Met Thr Phe Phe Ile Ile Ala Gln Ala Pro Glu Pro Tyr Ile Val Ile  
                   -65                  -60                  -55  
 Thr Gly Phe Glu Val Thr Val Ile Leu Phe Phe Ile Leu Leu Tyr Val  
                   -50                  -45                  -40  
 Leu Arg Leu Asp Arg Leu Met Lys Trp Leu Phe Trp Pro Leu Leu Asp  
           -35                  -30                  -25  
 Ile Ile Asn Ser Leu Val Thr Thr Val Phe Met Leu Ile Val Ser Val  
           -20                  -15                  -10  
 Leu Ala Leu Ile Pro Glu Thr Thr Thr Leu Thr Val Gly Gly Gly Val  
           -5                  1                  5                  10  
 Phe Ala Leu Val Thr Ala Val Cys Cys Leu Ala Asp Gly Ala Leu Ile  
                   15                  20                  25  
 Tyr Arg Lys Leu Leu Phe Asn Pro Ser Gly Pro Tyr Gln Lys Lys Pro  
           30                  35                  40  
 Val His Glu Lys Lys Glu Val Leu  
           45                  50

<210> 121  
 <211> 209  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -86...-1

<400> 121  
 Met Leu Ser Pro Thr Phe Val Leu Trp Asp Val Gly Tyr Pro Leu Tyr  
           -85                  -80                  -75  
 Thr Tyr Gly Ser Ile Cys Ile Ile Ala Leu Ile Trp Gln Val Lys  
           -70                  -65                  -60                  -55  
 Lys Ser Cys Gln Lys Leu Ser Leu Val Pro Asn Arg Ser Cys Cys Arg  
                   -50                  -45                  -40  
 Cys His Arg Arg Val Gln Gln Lys Ser Gly Asp Arg Thr Ser Arg Ala  
                   -35                  -30                  -25  
 Arg Arg Thr Ser Gln Glu Glu Ala Glu Lys Leu Trp Lys Leu Leu Phe  
           -20                  -15                  -10

114

Leu Met Lys Ser Gln Gly Trp Ile Pro Gln Glu Gly Ser Val Arg Arg  
       -5                          1                          5                          10  
 Ile Leu Cys Ala Asp Pro Cys Cys Gln Ile Cys Asn Val Met Ala Leu  
                           15                          20                          25  
 Glu Ile Lys Gln Leu Leu Ala Glu Ala Pro Glu Val Gly Leu Asp Asn  
                           30                          35                          40  
 Lys Met Lys Leu Phe Leu His Trp Ile Asn Pro Glu Met Lys Asp Arg  
                           45                          50                          55  
 Arg His Glu Glu Ser Ile Leu Leu Ser Lys Ala Glu Thr Val Thr Gln  
                           60                          65                          70  
 Asp Arg Thr Lys Asn Ile Glu Lys Ser Pro Thr Val Thr Lys Asp His  
 75                          80                          85                          90  
 Val Trp Gly Ala Thr Thr Gln Lys Thr Thr Glu Asp Pro Glu Ala Gln  
                           95                          100                          105  
 Pro Pro Ser Thr Glu Glu Glu Gly Leu Ile Phe Cys Asp Ala Pro Ser  
                           110                          115                          120  
 Ala

<210> 122  
 <211> 89  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -21...-1

<400> 122  
 Met Gly Ser Cys Ser Gly Arg Cys Ala Leu Val Val Leu Cys Ala Phe  
       -20                          -15                          -10  
 Gln Leu Val Ala Ala Leu Glu Arg Gln Val Phe Asp Phe Leu Gly Tyr  
       -5                          1                          5                          10  
 Gln Trp Ala Pro Ile Leu Ala Asn Phe Val His Ile Ile Ile Val Ile  
                           15                          20                          25  
 Leu Gly Leu Phe Gly Thr Ile Gln Tyr Arg Leu Arg Tyr Val Met Cys  
                           30                          35                          40  
 Thr Arg Cys Gly Gln Pro Ser Gly Ser Pro Gly Thr Ser Ser Ser Ser  
                           45                          50                          55  
 Ala Ser Thr Trp Lys Ser Val Ala Ser  
 60                          65

<210> 123  
 <211> 66  
 <212> PRT  
 <213> Homo Sapiens

<220>  
 <221> SIGNAL  
 <222> -19...-1

<400> 123  
 Met Lys Pro Leu Leu Val Val Phe Val Phe Leu Phe Leu Trp Asp Pro  
                           -15                          -10                          -5  
 Val Leu Ala Gly Ile Asn Ser Leu Ser Ser Glu Met His Lys Lys Cys  
                           1                          5                          10  
 Tyr Lys Asn Gly Ile Cys Arg Leu Glu Cys Tyr Glu Ser Glu Met Leu  
                           15                          20                          25  
 Val Ala Tyr Cys Met Phe Gln Leu Glu Cys Cys Val Lys Gly Asn Pro  
 30                          35                          40                          45  
 Ala Pro